

The Journal of Parasitology

Volume 7

SEPTEMBER, 1920

Number 1

WOHLFAHRTIA VIGIL (WALKER) AS A HUMAN PARASITE (DIPTERA—SARCOPHAGIDAE)

E. M. WALKER

DEPARTMENT OF BIOLOGY, UNIVERSITY OF TORONTO

On June 15, 1919, a Toronto surgeon called the writer by telephone and described a case of boil-like sores in an infant, from some of which he had removed small, whitish maggots. On my request to see the latter, the child and its mother were brought by the surgeon to my house, where the larvae were removed.

The patient was a female infant, two weeks old, in fair condition, though irritable almost to the point of exhaustion. The sores consisted of twelve somewhat swollen, inflamed areas, about one to two and a half centimeters in diameter, scattered over the front of the neck and arms, palms and chest. One of the palms was particularly red and swollen. On each sore there was a minute and very inconspicuous external opening. From these sores nine dipterous maggots were extracted, each from a different sore, although as some of the sores had been previously opened, it is possible that one or two of them may have contained more than a single larva. Another was removed from a spot on the shoulder on the following day, according to the mother's statement, after which operation the child recovered rapidly.

The larvae, all but two of which were more or less injured in the operation, were preserved in alcohol. They vary in length from 2.5 mm. to 4 mm., but this variation is partly due to the contraction of the injured specimens and the considerable extension of the largest uninjured one. All appear to belong to the same stage, though there is some difference in the individual size of the head segment and mouth-parts.

The sores had been noticed by the mother for the first time on the evening of June 13. The mother states that the baby had not been sleeping out of doors on that day but that the front door had been left open, so that the fly must have entered the house to deposit its young. The house is in a fairly densely populated section of North Toronto, but is less than half a mile from the ravine and wooded country in the vicinity of Reservoir Park.

A second case, very similar to the above, was brought to my notice on June 23 by a Toronto physician. The child, whose home was in West Toronto, was admitted to the Sick Children's Hospital on this date, and I visited the hospital in the afternoon. The patient was a female infant, eight weeks old, well grown and well nourished. There were fourteen lesions distributed upon the front of the neck, chest and anterior surface of the arms. They were of somewhat larger size than those of the first case, averaging about 2 cm. in diameter. Those on the neck were particularly swollen and inflamed. The mother had not observed anything wrong with the baby until three days previous to its admission to the hospital, when pimples appeared on the neck. On the twenty-second "worms were seen to come from the pimples."

Each swelling had a round or elliptical opening about 3 mm. in diameter and from some of these larvae were squeezed out. Usually there was a single larva in each swelling but from one of them three larvae were expelled.

When I visited the hospital some eight or nine larvae had been removed and placed upon raw beef in an incubator at body temperature. The surgeon was just completing the extraction of the remainder (about ten), which were kindly given to me for investigation. I placed them upon some raw beef in a test tube and took them home. According to the hospital record these larvae varied in length from about 5 to 15 mm. The latter measurement appears to me excessive unless the specimens were fully extended.

On the following day (June 24) the larvae had greatly increased in size and were very active, feeding on the underside of the meat. One, however, had crawled a little way up the side of the tube and, being unable to return, had died. This was preserved in 70% alcohol. On the next day the larvae were apparently full grown, measuring about 17 or 18 mm. in length (exact measurements were not made). The meat upon which they had been feeding had become extremely putrid, so I introduced a fresh piece, but they did not touch it.

On the morning of June 26 I removed the larvae and meat to a half pint jar, which I partly filled with slightly moist earth to a depth of about four inches. I then observed that only four larvae were present, all of them evidently full grown. No trace of the others remained. They could not have escaped from the tube, which was kept upright and plugged with cotton wool, and it would therefore appear that they had been devoured by the survivors. This was unfortunate, as I had intended to preserve one or two of the full grown larvae.

On the same day I took the jar containing the larvae to DeGrassi Point, Lake Simcoe, where on the following morning they had commenced to burrow into the earth. On June 28 two of the larvae were still at the surface but were becoming shorter and more oval in form. On the 29th all were beneath the surface, but two could be seen through the side of the jar and were moving somewhat actively. After this they disappeared from view.

On July 4 I dug them up. All had transformed to puparia and were at or near the bottom of the jar. I placed them upon somewhat drier earth in a breeding cage.

On July 18, about 9 p. m., I looked into the cage and saw four soft-looking pale gray flies with wings not yet expanded, crawling about the sides of the cage. One of them, while walking over the mosquito netting which covered the front of the cage, thrust its ptilinum through one of the meshes and had to be pushed back. The wings were not fully expanded until 11 a. m.

I then smeared some sweetened milk on the netting, which they devoured greedily. By the afternoon they were very active, running up and down the walls of the cage. They were readily recognized as Sarcophagids but had, to me, an unfamiliar appearance. I kept them until July 24, feeding them upon sweetened diluted milk and jam, which they took at all times of the day very readily, but on this date I found that two had died and the others were somewhat sluggish, so that, relinquishing the hope of obtaining fertilized eggs I killed the remaining two. Up to this date they had been very active.

The larvae that were kept at the hospital were reported to have "grown to two or three times their former size" on June 26, but were unfortunately destroyed when the meat upon which they were feeding became very putrid.

As in the first case the child recovered rapidly after the removal of the larvae, no secondary infections having developed.

The four flies obtained consisted of one male and three females and were readily determined from Mr. Aldrich's admirable monograph as *Wohlfahrtia vigil* (Walker) and Mr. Aldrich, to whom I sent one of the specimens, kindly confirmed my determination.

The genus *Wohlfahrtia* was erected by Aldrich (1916) for certain Sarcophagid flies formerly included in the genus *Sarcophila* and the type species *W. magnifica* (Schiner) (*Sarcophila wohlfahrti* Portchinsky) has long been known as a parasite of man and various domestic animals, particularly in Russia, having been regarded as the European analogue of the Screw-worm Fly (*Comptosia macellaria* Fabr.) of the warmer parts of America. Such habits are unknown, however, for the other European species of *Wohlfahrtia*, one of which, *W.*

meigenii, occurs also in western North America and is so closely related to the eastern species, *W. vigil*, as to be perhaps only a race of this species (vide Aldrich, 1916).

Nothing has been hitherto known of the larval habits of the North American species of *Wohlfahrtia*. Concerning *W. magnifica* several valuable papers were published between the years 1874 and 1876 by Joseph Portchinsky of St. Petersburg. A review of this work by Osten Sacken appeared in 1877 and a copy of this was very kindly sent to me by Mr. Aldrich. In this review is the following paragraph:

"In 1875-76 Portchinski published an elaborate paper, entitled 'Materials for the natural history of the flies which, in their larval stage, cause diseases among men and animals' (Trudy, etc., vol. ix, p. 3-180, with three plates). A condensation of a portion of this paper concerning *Sarcophila wohlfahrti* was published in the Horae Soc. Ent. Ross., vol. xi, 1875, pp. 123-180, in German under the title 'Krankheiten welche im Mohilewschen Gouvernement von Larven von *Sarcophila wohlfahrtia* entstehen und deren Biologie.' In 1884 a monographic essay on *Sarcophila wohlfahrti* appeared (Horae, etc., vol. xviii, p. 247-314, with 33 woodcuts), containing some new observations and comparative descriptions of this fly and its next relatives."

On Mr. Aldrich's suggestion and through the kindness of the Secretary of the Smithsonian Institution I obtained the loan of a copy of the former volume, in which Portchinsky describes a number of cases of human myiasis, caused by *Wohlfahrtia magnifica* (*Sarcophila wohlfahrti*), chiefly in children under 13 years of age. In all these cases the larvae are described as feeding gregariously upon the mucous membrane and underlying tissues of the ear, nose, gums or even the eye, or in one case an eczematous scalp; but no mention is made of the larvae ever penetrating the healthy skin, as must have occurred in the two cases of infection by *W. vigil*. In the case of a five year old boy who had a copious discharge of blood and pus from the nose there were six round openings on the upper lip, close to the nostrils, but these were probably not points of entrance as they appeared to communicate with the frontal sinus by way of the nasal cavity. The larvae would frequently come to the surface through these passages and would sometimes protrude considerably from the openings. In the cases of infection by *W. vigil* the scattered distribution of the lesions, as well as the penetration of the skin by the larvae, seem to indicate a distinctive habit, but the apparent difference from *W. magnifica* in this respect may be due merely to the difference in the ages of the hosts; the healthy skin, except that of very young infants, being perhaps impenetrable by the young larvae of either species.

In this connection it may be worth while to record that a farmer residing near Port Sydney, Ont., who is also a keen naturalist, told me that a few years ago he had suffered from severe pains in the nose, accompanied by a sensation as though something were creeping within it, and that, after a violent sneezing fit, a large maggot had dropped out; after which the trouble subsided. The capture of a specimen of *Wohlfahrtia vigil* in this locality, by Mr. N. K. Bigelow, indicates the possibility of the larva having belonged to this species.

While it is impossible to prove that the larvae from the first case belong to the same species as those from the second, the clinical features of the two cases were so very similar that I have no hesitation in considering them both to belong to *Wohlfahrtia vigil*, in spite of certain differences which are described below. These differences are not surprising in larvae which represent different stages of development; they are in fact less than those which occur in *W. magnifica*.

Figures 1 and 2 represent the largest of the larvae taken from the first case. It measures 4 mm. in length but is fully extended and agrees in all other respects with the smaller larvae from the same case. Figure 3 is a ventro-lateral view of the head of the same larva.

The two lobes into which the upper part of the pseudocephalon (cephalic segment) is divided appear somewhat less prominent than in the 3 lines long larva of *W. magnifica* figured by Portchinsky (see Osten-Sacken, 11. pl. 4, fig. 1) and the mandibular sclerites (lateral hooks) are shorter and blunter. The anterior spiracular processes are much broader than long and bear 9 to 10 minute spiracular papillae, whereas that of *W. magnifica*, figured from a 2½ lines long larva, is much longer than broad and terminates in only 4 papillae, which are long and capitate. The posterior spiracles in *W. vigil* at this stage, which is probably the second, have only two openings.

The spinules of the trunk segments are very much smaller and more restricted in distribution than in *W. magnifica*. Those of the second segment (strictly the united second and third) form a narrow ring at the front margin and are so minute as to be invisible except under high magnification (Fig. 3). Those of the other segments are larger and visible under much lower powers, but are nevertheless very minute. They are arranged, for the most part, in small, subtransverse groups of two to four. Those of the third, fourth and fifth segments are arranged in a single ring at the anterior margin of each segment, that of the third segment very narrow, those of the fourth and fifth increasing in width to nearly one third the width of the latter segment. On the remaining segments the rings of spinules are similar on the dorsal surface, but more irregular ventrally and laterally. On the ventral surface they form a transverse patch, occupying the

anterior third or more of the length of the segment and enclosing a transversely elongate bare area. These patches are confluent with a narrow band along the caudal margin of the next preceding segment, and the latter bands are continuous or subcontinuous laterally with a narrow strip of spinules along the front of the lateral fold.

In the smallest larva of *W. magnifica* figured by Portchinsky the spinules are much larger and not arranged in small groups. The spinulose bands are much more extensive. That of the second segment is much wider though described as narrow, and consists of very small spinules. The third segment is described as being bare in the middle, but provided with spinules on the front and hind margins; the third segment is entirely covered with spinules below, except a narrow bare band on the hind margin and a likewise bare, triangular, elongate space, which lies about the middle of the segment. Segments 4 to 6 are similar to segments 5 to 7 in *W. vigil* except that the spinulose bands are much broader and the enclosed bare area is divided transversely by a row of spinules. The two following segments are wholly covered with spinules, except for a narrow bare area on each and the lateral folds. Segment 9 is armed with spinules only on the anterior half, with only a few rows of spinules on the posterior half. Segment 10 presents almost the same pattern, except that the spinulose band on the front margin is narrower and the remainder of the segment almost naked. The last segment is almost entirely naked, being provided with spinules only on the middle of the front margin and at the base of the two anal papillae.

The single larva of *W. vigil* that was preserved from the second case (Figs. 4-6) belongs to a later stage than those from the first case, and though only 7 mm. long, probably shows the characteristics of the mature larva. The pseudocephalon is similar to that of the larva described above, except that the outline as seen from below is nearly square, the sides being parallel and the emargination of the front narrower. The mandibular sclerites are somewhat blunter and less curved. The posterior spiracles have three slits, like those of *W. magnifica* and other Sarcophagids. The anterior spiracular processes are like those of the earlier stage, being very short and broad, with an arcuate margin bearing 9 papillae. The spinules are wholly absent, being represented only by minute granulations which are invisible except under high magnification. They are difficult to see in the single larva preserved, but can be readily distinguished in the puparia, in which their arrangement is seen to be essentially the same as that of the spinules in the young larva (*cf.* Figs. 1 and 7).

It is worthy of note that whereas in this species the spinules are lost during development, in *W. magnifica* according to Portchinsky, they increase in both number and size, as shown in two successive

instars described and figured on plate III of the work cited above. Portchinsky believes that the great development of spines in this species is connected with its parasitic life. If this be true it would appear probable that the parasitic habit is abnormal in *W. vigil*, in which the cuticle is even less spiny than in many muscoid larvae that develop in dead organic matter.

Puparium. (Fig. 7). Length 9 to 10 mm., diameter slightly less than half the length; the ends slightly flattened; segments marked with narrow, slightly roughened bands having essentially the same arrangement as the spinules of the young larva (Fig. 1); pocket enclosing the posterior spiracles with a slightly raised margin.

In order to examine the cephalo-pharyngeal sclerites, these were removed from the inner surface of one of the empty puparia, and are shown in Figs. 8 and 9. The pharyngeal sclerites (*ps*)* are united in front, both dorsally and ventrally. Each is prolonged behind into three processes, a ventral, continuous with the floor of the pharynx, widening somewhat caudad, with a narrow, thinly chitinated area next to the hind margin, a dorsal, slightly longer, directed ecto-caudad and somewhat expanded distally, and a lateral, considerably longer than either of the others, somewhat sinuate, with slender apices. The notch between the ventral and lateral processes is somewhat deeper than that between the middle and dorsal processes. The hypostomal sclerite (*hs*) consists of two subtriangular, ventrolateral plates, united by a ventral arch with the concavity caudad. Behind and above the arch is a pair of slender, rod-like processes of the pharyngeal sclerite (*r*), and in front of the arch is a pair of small, short sclerites. The mandibular sclerites (*ms*) are not much elongated, and are rather blunt and but little decurved. Their proximal half is stout and bears a prominent ventral tooth, with which is connected the small dental sclerite (*ds*).

The female adults measure 10.5 to 11 mm. in length, the male 13 mm. The male from Port Sydney, Ont., is 12 mm. long.

REFERENCES CITED

- Aldrich, J. M. 1916.—*Sarcophaga and its Allies in North America*. Thos. Say Foundation, pp. 25-30.
 Hewitt, C. G. 1914.—*The House-fly, Musca domestica* Linn. Cambridge Zool. Series, 382 pp.
 Osten Sacken, C. R. 1887.—On Mr. Portchinski's publications on the larvae of Muscidae including a detailed abstract of his last paper; Comparative biology of the necrophagous and coprophagous larvae. *Berliner Ent. Zeitsch.*, 31, no. 1.

* The terminology followed here is that of C. G. Hewitt (1914:134).

A NEW CYSTOPHOROUS CERCARIA FROM CALIFORNIA *

W. W. CORT

School of Hygiene and Public Health, Johns Hopkins University

AND

ELINOR B. NICHOLS

Berkeley, California

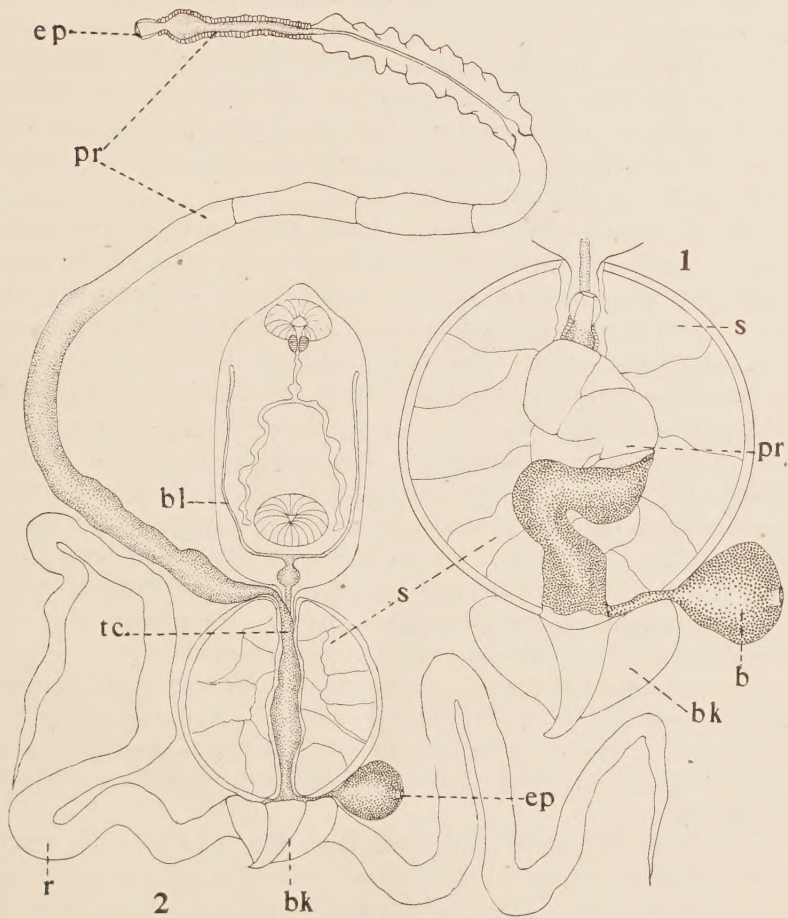
A number of specimens of *Physa occidentalis* collected from Lake Temescal, a city reservoir near Oakland, California, were heavily infested with a new cercaria, to which the name *Cercaria californiensis* spec. nov. will be given. The infection was localized in the digestive gland of the snail. At the time that the examinations were made (September to December) there were found in the infected snails only rediae which contained cercariae. No sporocysts or rediae containing rediae were found.

The rediae of this species vary considerably in size, ranging from 0.5 mm. to 1.6 mm. in length and have a width equal to about one-fifth or one-fourth the length. They are elongate sacs, slightly narrowed at the anterior end, and are without lateral projections. The body walls of a few of these rediae contain a pale orange pigment, the others being quite transparent. Contrary to what is usually observed in pigmented parthenitae, the pigment in this species appears in the immature rediae, the older ones being always unpigmented. The pharynx is small and the intestine extends about one-fourth of the total length. No birth pore is present. When the rediae are first freed from the digestive gland of the snail host they contract and expand actively for a few minutes, but are unable to move from place to place. Cercariae and developing germ balls fill the body cavity of the rediae from the posterior end to the region just behind the pharynx. The number of fully developed cercariae contained within a redia varies from eight to fifty. This great variation in number can easily be explained since in the absence of a birth pore all the cercariae which develop must remain within the redia.

The body of *Cercaria californiensis* (Fig. 2) is very small, having a length varying from 0.12 mm. to 0.18 mm. according to the state of contraction. The attachment between the tail and the body is weak, and is usually broken soon after the cercaria is freed

* A publication from the Department of Medical Zoology of the School of Hygiene and Public Health of Johns Hopkins University and from the Department of Zoology of the University of California.

from the redia for study. While still attached to the tail the cercaria was never seen to move about, but when it had broken loose it could locomote slowly by the use of its suckers. The body cannot be contracted into the tail, but always keeps the relation to the tail shown in figure 1 while within the redia and, when freed from the



redia, the position shown in figure 2. The size and position of the various organs of the body can be made out readily from figure 2. The ventral sucker is slightly larger than the oral and is in the posterior third of the body.

The mouth of *C. californiensis* is on the ventral side a little distance back of the pointed anterior tip. There is no prepharynx.

The pharynx is small and is followed by an esophagus of medium length (Fig. 2). The intestinal ceca extend back to the level of the ventral sucker.

Of the excretory system only the parts of the bladder were distinguished (Fig. 2, *bl*). The undivided portion of the bladder has a globular enlargement containing the highly refractive concretions which are so commonly found in the excretory bladder of cercariae and agamodistomes. It is connected by a narrow tube with the excretory tubes of the tail. The bifurcations of the bladder were traced forward to a point just back of the pharynx. The region of the bifurcations nearest the enlargement and the tube leading into the tail also contain the concretions. The peculiar relations of the excretory canals in the tail will be discussed later.

The tail of *C. californiensis* is very remarkably differentiated. Figure 1 shows its appearance when the cercaria is within the media or just after it has been forced out. In this condition the tail appears as a large firm cuticular sphere (Fig. 1, *s*) with two short projections at its posterior end (Fig. 1, *bk* and *b*). One of these projections has the form of a cuticular beak (*bk*). It is entirely transparent and is divided into a central area which is pointed posteriorly and two lateral areas. The beak is so transparent that nothing can be seen of the ribbons (Fig. 2, *r*) which are later extruded from the lateral areas. To one side of the beak is a bulb (Fig. 1, *b*) with an opening on its outer surface. This bulb is loaded with excretory concretions. The sphere has a thick cuticular wall and is very difficult to break when pressure is applied on the cover glass above it. The sphere has a central column of living material (Fig. 2, *tc*) along which is folded a very complex cylindrical structure. The portion of the sphere between the central column and the wall is highly vacuolated. The posterior region of this body is directly connected with the column of the sphere, and a tube from the excretory bladder of the body extends down into this column.

Soon after the cercaria is freed from the media two flat transparent cuticular ribbons (Fig. 2, *r*) are extended out laterally from the sides of the beak. These ribbons are pointed and have no apparent structural differentiation. At the same time there is slowly protruded from the place where the body joins the tail sphere the long cylindrical projection which, in the condition indicated in figure 1, is folded along the column of the sphere (Fig. 1, *pr*). This structure, which will be called the excretory projection, is protruded until it has a length more than three times that of the body of the cercaria (Fig. 2, *pr*). The excretory projection is a hollow cuticular cylinder. The proximal half of this cylinder is undifferentiated, has a very

thin wall and contains excretory concretions. Beyond the middle the excretory projection appears to be jointed externally. At about the beginning of the distal third of the excretory projection the cuticular wall becomes much thicker, narrowing the lumen. In the first half of this region the greatly thickened cuticula appears to be ruffled. Distal to this region the lumen widens slightly and the cuticula appears to be fluted. The excretory projection ends with a dilation followed by a cup-like structure with a large opening at its end. The first half of the excretory projection and the last fluted part contain excretory concretions. The excretory projection is broadly attached to the posterior part of the column of the tail (Fig. 2, *tc*). The differentiations of the excretory projection are shown in figure 2.

The presence of excretory concretions in various parts of the tail of *C. californiensis* indicates the relation of these parts to the excretory system. There are two openings of the excretory system in the tail (Fig. 2, *ep*), one on the outer surface of the excretory bulb, and the other at the end of the excretory projection. The excretory bulb is directly continuous with a tube which runs from the bladder of the body down the column of the tail. The lumen of the excretory projection is also directly connected with this tube. These connections are indicated by the presence of the concretions in the various structures. Movements of the concretions could be followed from the tube in the column of the tail into the excretory bulb. Excreta from the body of *C. californiensis* can escape after passing down the column of the tail-sphere either by the pore of the excretory bulb or after passing along the excretory projection from the opening at its end.

Apart from the relation to the excretory system described above, it is difficult to suggest functions for the various parts of the tail of *C. californiensis*. The function of the tail of a cercaria is evidently to aid it in that period of activity, usually including a short period of free life, from the time it leaves its parthenita until it is settled in its secondary intermediate host, its final host, or is encysted in the open waiting to be taken into its final host. We have no direct evidence in regard to the further development or activities of *C. californiensis*. There are certain things, however, which it is perfectly evident that this cercaria does not do. The absence of a stylet and cephalic glands and the weak muscular development of the body of the cercaria make it evident that it does not penetrate actively into the next host. The absence of cystogenous glands in the body and the inability to withdraw into the vesicle of the tail show that it does not encyst in the open and wait to be taken into its next host. Finally

the fact that the redia has no birth pore, that the cercariae accumulate in the redia and that they are practically incapable of locomotion in the open, makes it very probable that this cercaria never leaves its intermediate host and continues its development in some vertebrate which feeds on this host.

There is nothing in such a life cycle which would make it possible to explain the complex structure of the tail of *C. californiensis* in terms of function. The fact that in three closely related forms *C. cystophora* (Wagener, 1866), *C. sagittarius* (Ssinitzin, 1911:15-19), and *C. yoshidae** (Yoshida, 1917) the body of the cercaria can be withdrawn into the tail for protection suggests that this is the primary function of such a vesicle as the sphere of *C. californiensis*. A change in life cycle which eliminated the free stage and therefore the importance of this function may have led to the loss of the power of withdrawal into the tail vesicle without the loss of that structure. It is still more difficult to determine any function for the appendages of the tail. Ssinitzin, (1911:18) suggests that the various appendages of the tail of *C. sagittarius* hold the tail vesicle, which forms a cyst containing the cercaria, in position in the digestive tract of the final host until the cercaria has had a chance to get out of its cyst. Such a function for the excretory projection and ribbons of *C. californiensis* seems very improbable since in the first place this cercaria does not become encysted in the vesicle of the tail and in the second place the body is so weakly attached to the tail that even a slight pull will break the connection. It is, of course, possible that these appendages may also represent structures which have lost their function through modifications of the life cycle. Finally it is very probable that structures may develop in connection with the evolution of such a diversified organ as the cercaria tail which have no function whatsoever.

C. californiensis belongs to a small group of cercariae which Ssinitzin (1911:20) named the Cystophrous Cercariae. He characterizes this group as containing cercaria which possess a vesicular tail with various appendages. The first cercaria of this type to be described was *Cercaria cystophora* which Wagener (1866) reported from *Planorbis marginatus*. Another cercaria which probably belongs to this group was described by Sonsino (1892) from *Cleopatra bulimoides* as *C. capsularis*. Sonsino's original description is hardly sufficiently detailed to make the relationship of this species clear. The same cercaria is later described in the immature state by Looss (1896). These two descriptions taken together make it pretty certain that

*I use this name to designate the species which Yoshida describes as *Cercaria* F.

this form belongs to the cystophorous cercariae, but do not give enough data to make a detailed comparison possible. Pelseneer (1906) described two marine representatives of this group, *C. appendiculata* from *Natica alderi* and *C. vaullegeardi* from *Trochus cinerarius*. Later Ssinitzin (1911: 15-21) described two more marine cystophorous cercariae, *C. sagittarius* from *Cerithium exille* and *C. laqueator* from *Rissoa venusta*. Finally there should be added to this group *C. yoshidae* (Yoshida, 1917) from *Melania libertina* from Japan and *C. californiensis*. While the cystophorous cercariae vary considerably in structural details, they have so many important points in common, that we consider that they form a natural group.

All the cystophorous cercariae except *C. vaullegeardi* (Pelseneer) develop in rediae. Mother sporocysts in which the rediae develop have been described for two species, *C. sagittarius* Ssinitzin (1911: 15) and *C. cystophora* (Wagener, 1866). In none of the species were rediae which contained rediae found and the descriptions of the two species of which the mother sporocysts were found, indicates that such a stage is not present in the life histories of the members of this group. The rediae are much alike having no locomotor appendages, a very small pharynx and a voluminous digestive tract varying from one-fourth the length of the redia in *C. californiensis* to two-thirds its length in *C. yoshidae*.

The body of the cercariae in all cystophorous cercariae is very small and the primordial adult characters are very slightly developed. This is indicated by the small size, the lack of clear differentiation of the organs and the short distance between the ventral sucker and the posterior end. The bodies of these cercariae lack entirely all adaptive larval structures for penetration and encystment, i. e., stylet, cephalic glands and cystogenous glands. The digestive system in those forms for which it has been described is much like that of *C. californiensis* (Fig. 1), except that the length of the intestinal ceca varies. The excretory bladder is y-shaped.

The tails in these forms differ considerably in detail, but are all built on the same fundamental plan. There is present in each species a central vesicle or sphere as we have called it in *C. californiensis*. This is a firm structure with a thick cuticular wall which was evidently developed to function as a cyst into which the cercaria can be withdrawn into the cavity of the sphere as in *C. sagittarius*, *C. cystophora*, *C. yoshidae*. In regard to *C. laqueator* and *C. californiensis* the statement is made that the body of the cercaria cannot be withdrawn into the vesicle of the tail. It is evident that this ability of the cercaria to withdraw into the tail would be a very important protective measure

at the time of entrance of the cercaria into its next host. Besides the central vesicle there is found in every species of this group a protractile appendage comparable to the structure we have called the excretory projection in *C. californiensis*. This structure differs greatly in the various species and in some is not sufficiently described for comparison. The various other appendages which are found on the tails of the Cystophorous cercariae are so remarkably varied and grotesque that it is not only impossible to get an idea of what their function may be, but also there seems to be no homologies which can be worked out between them.

Further development is known for only one of the cystophorous cercariae, *C. cystophora*, which develops into *Halipegus ovicaudatus* which is found in the mouth cavity of the European frog. Ssinitzin (1905 and 1907) finds the agamodistine stage of this species free in the body cavity of the nymph of the dragon-fly *Calopteryx virgo*. On this account he suggests (Ssinitzin, 1911:21) that the life cycles of the other members of this group will probably follow the same course. This deduction does not seem to us to necessarily follow since a fundamental modification of life cycle might come to a species, and yet the structural adaptations for the previous mode of life might be retained. Certainly in the case of *C. californiensis* there could hardly be a secondary intermediate host, since it seems very improbable that this cercaria ever leaves its molluscan intermediary.

SUMMARY

1. *Cercaria californiensis*, a new species of cercaria from *Physa occidentalis*, has a very small body which shows no adaptive larval characters and but slight development of primordial adult characters.

2. The tail of this species is very remarkably differentiated, consisting of a central cuticular vesicle, the sphere, and various projections and appendages.

3. It is not possible to explain the functions of the parts of this tail in terms of activities of the cercaria since the cercaria probably never leaves the redia within its intermediate host.

4. *Cercaria californiensis* belongs to a natural group, the cystophorous cercariae which are characterized by a small, very slightly differentiated body and an extremely complex tail consisting of a central cuticular vesicle, with various appendages.

LITERATURE CITED

- Looss, A. 1896.—Recherches sur la faune parasitaire de l' Egypte. Première partie. Mem. l'institute Egyptien, 3:1-252; 16 pls.
Pelseneer, P. 1906.—Trematodes parasites de mollusques marins. Bull. scien. France et Belg., 40:161-186; 5 pls.

- Sonsino, P. 1892.—Studi sui parassiti molluschi di acqua dolce dintorni di Cairo in Egitto. Leuckart's Festschr., pp. 134-147.
- Ssinitzin, D. Th. 1905.—Studies on the life-cycle of trematodes. The Distomes of the Fish and Frogs in the Environs of Warsaw. (Russian.) Mem. soc. nat. Varsovie biol., 15:1-210, 6 pls.
- 1907.—Observations zur les métamorphoses des Trématodés. Arch. zool. expér et génér., 37, notes et rev., pp. 21-27.
- 1911.—The Parthenogenetic Generation of Trematodes and their Progeny in the Molluscs of the Black Sea. (Russian) Records Imp. Acad. Sci., (8) 30:1-127; 6 pls.
- Wagener, G. R. 1866.—Ueber Redien und Sporocysten Filippi. Arch. Anat., Physiol., u. wissenschaft. Med., 145-150. 6 pls.
- Yoshida, S. 1917.—On the Cercariae from Melania in Japan. (Japanese) Dobutsu Jaku Zasshi (Zoological Magazine) vol. 29, no. 342.

ON THE NATURAL OCCURRENCE OF HERPETOMONADS (LEPTOMONADS) IN THE BLOOD OF
A FISH, *DENTEX ARGYROZONA*, AND
ITS SIGNIFICANCE

H. B. FANTHAM

Professor of Zoology, University College, Johannesburg,

AND

ANNIE PORTER

Parasitologist, South-African Institute for Medical Research

During the years 1908-1916, we were conducting researches, individually and in collaboration, on the life-cycles of Trypanosomes, Crithidia and Herpetomonas and their significance in the evolution of disease. After working on the life-histories of the Protozoa used by us, we were able to show, by direct experiment, that Herpetomonads and Crithidia could be inoculated into or fed to vertebrates and produce therein pathogenic effects resembling those of leishmaniasis. We also found natural occurrences of herpetomonads in mice, and in 1916 summarized our experimental conclusions to date, when war-work on the diagnosis of protozoal diseases completely stopped our further progress. Recently (Jan.-Feb., 1919), while working at the St. James Marine Aquarium, near Cape Town, we were agreeably surprised to find a Herpetomonas in the heart blood of a freshly killed silver-fish, *Dentex argyrozona*. Subsequently, the same organism was found in small numbers in the blood and organs of three more Dentex, a total of 4 out of 41 examined containing the Herpetomonas, but in each case the infection was scanty.

As no mention of such a parasite in the blood of fish can be found in the literature available, we propose to describe the Herpetomonas, and to name it *Herpetomonas denticis*. It is true, that, morphologically, it is somewhat difficult to separate this species from others, but the occurrence of physiological species or races is known, and such may be the case here. New and unexpected methods of research in future may shed further light on physiological species; at present we think that we are justified in giving a separate name to the Herpetomonas of *Dentex argyrozona* for purposes of reference. We regret that, as a result of our comparative studies, we are unable to accept a biflagellate character as diagnostic of the genus Herpetomonas, and it is also unfortunate that *Leptomonas gracilis*, the type species of Leptomonas, has not been studied by modern methods. We therefore accept Butschli's (1884) definition of the genus Herpetomonas.

As indicated in the introduction, the herpetomonads were found in the blood and organs of freshly killed silver-fish, *Dentex argyrozona*. Fresh preparations of the blood taken direct from the heart were examined both directly and by the aid of the paraboloid condenser, and stained preparations of both heart blood and internal organs of the fish were examined. Fixation by exposure to osmic acid vapor and formalin vapor followed by absolute alcohol, and direct wet fixation with Schaudinn, Carnoy or Bouin-Duboscq fluids were tried, while Giemsa, Delafield's hematoxylin and iron-hematoxylin stains were used. The most useful preparation was one fixed with osmic vapor and absolute alcohol and stained with Giemsa's solution.

DISTRIBUTION OF THE PARASITE IN THE HOST

At no time was *Herpetomonas denticis* abundant in any fish that we examined. It was seen very rarely and with difficulty in life. The parasites were most often found in stained smears made from the heart blood of the host. A few were found in the spleen and liver. Other organs were rarely infected, though a few parasites have been observed in a preparation from the kidney of one host. Intracellular forms have not been observed.

The herpetomonads were present both in the flagellate and the non-flagellate phases. As a general statement, flagellate forms were more common in the heart blood and non-flagellate forms in preparations of organs such as the spleen.

No herpetomonads were seen in the gut of any of the *Dentex*, though careful search was made. No marked pathologic effects on the hosts were observed.

MORPHOLOGY

The flagellate form of *Herpetomonas denticis* is small, the body measuring from 5 to 24μ long and 1.5 to 2.5μ broad (see photomicrographs). The variation in length is rather great, the short-bodied forms being apparently younger. The flagellum is often longer than the body, especially in young flagellate forms, as, for example, in a parasite whose body length was 7.5μ and length of free flagellum was 16μ . The posterior (non-flagellate) end of the body was sometimes pointed, at other times rounded.

The general cytoplasm was almost homogeneous, though in some specimens a finely alveolar structure was seen. Chromated granules may be seen in some specimens.

The nucleus was karyosomatic in some cases, and finely granular in others, the structure varying as we have pointed out previously, with the degree of activity of the cell-life. Prior to periods of great

multiplicative activity, the nucleus usually becomes finely granular in a flagellate, and such changes can be observed in the living organism under favorable conditions.

The blepharoplast, or kinetic nucleus of some authors, is distinct and often bar-like, but sometimes is slightly curved or rounded, the latter appearance probably being due to an end-on view. The organella may be surrounded by a less deeply staining area of cytoplasm.

The flagellum arises in the neighborhood of the blepharoplast but not from it. The root of the single flagellum is usually well marked.

The non-flagellate stages are small, oval or somewhat pyriform bodies, possessing a nucleus and distinct blepharoplast. The small oval or leishmaniform parasites measure 2.5 to 4.5 μ by 1.5 to 2.5 μ . Larger forms, elongating into flagellates though still lacking a flagellum, may be somewhat longer and broader.

Multiplication by fission occurs among both flagellate and non-flagellate forms. Division begins in the blepharoplast and is followed by division of the nucleus. Longitudinal fission was seen in flagellate parasites and in several intermediate elongating forms.

The occurrence of division shows that the herpetomonad could increase in numbers in the Dentex, and so was more than a mere conservation of the organism.

SIGNIFICANCE OF NATURAL HERPETOMONADS IN VERTEBRATES

The significance of the findings of herpetomonads in the blood of representatives of most classes of vertebrates is most important. The published results of our personal work on the life-histories of Herpetomonads and Crithidia have been strongly indicative that leishmaniasis—such as kala-azar, dermo-mucosal and dermal leishmaniasis—were really due to herpetomonads being able to live in the blood of vertebrates. Further, we have conducted experiments on the inoculation and the feeding of herpetomonads and a few crithidia to all classes of vertebrates with positive results. Laveran and Franchini have performed similar experiments and Laveran has shown that Leishmania can be inoculated into cold-blooded vertebrates. These various experiments have been carefully discussed recently by Laveran in his monograph on "Leishmanioses." We have had the good fortune to find herpetomonads occurring naturally in the blood of mice and of Dentex.

At present, herpetomonads have been found occurring naturally in the blood of the following vertebrates.

(1) Man. In 1913 a herpetomonad was described by Franchini from the blood and internal organs of man. Unfortunately, the name *Haemocystozoon brasiliense* was given to the organism. An allied parasite was recently found by M. Léger in French Guiana, herpeto-

monad and trypanosome forms being seen. It should also be mentioned here that herpetomonad flagellate forms of *Leishmania* have been seen in man.

(2) Mice. Herpetomonads were seen in the blood of Gambian mice by Dutton and Todd in 1903, while Fantham and Porter published similar observations on the natural occurrence of herpetomonads in the blood of English mice in 1915, these having been seen from time to time during the previous six years.

(3) Pigeons. Natural infections of these birds by a herpetomonad was found by Edmond and Etienne Sergent in 1907 in Algeria.

(4) Reptiles. Natural infection of geckos in Algeria with herpetomonads in the blood was found in 1914 by Sergent, Lemaire and Senevet. The Herpetomonads of geckos was also found by Chatton and Blanc in Tunis in 1918. Marcel Léger in 1918 found herpetomonads in the blood of small lizards belonging to the genus *Anolis* in Martinique.

(5) Fishes. A herpetomonas occurring in the blood and internal organs of *Dentex argyrozona* is now recorded by us.

As before mentioned, we were able to produce herpetomoniasis experimentally in all groups of vertebrates from Pisces to Mammalia. From the foregoing list, it will be seen that herpetomonads in nature have a similarly wide distribution in vertebrates, having been found in the blood of representatives of all the great groups of vertebrates except Amphibia, in which they will doubtless sooner or later be detected. However, it should again be pointed out that in no case in vertebrates is the flagellate form numerous, the leishmaniform phase being the one most seen, and relatively few vertebrates seem to be infected. In some cases it may be necessary to culture the blood in order to detect the parasite. Artificially induced herpetomoniasis resembles visceral leishmaniasis in its insidious onset and pathogenic effects such as feverish attacks, splenic enlargement often accompanied by hepatic enlargement, emaciation, progressive anemia and leucopenia.

The mode of entry of the herpetomonads into the blood of the *Dentex* examined by us is, unfortunately, not certain. It may be due to the inoculative action of an ectoparasite such as a leech. In the case of fresh-water fishes, herpetomonads might be introduced into their blood by aquatic biting Hemiptera such as *Nepa*. The entry of a natural intestinal parasite into the blood from the gut seems to be excluded, as, in every case, we carefully examined the gut contents of the fishes dissected by us, but found no indication of the presence of a *Herpetomonas* as a natural parasite in the guts. We may remark here that we always made a practice of examining before use the

dejecta and blood of the vertebrate animals subsequently used by us in our previous experiments on induced herpetomoniasis, and on no occasion did we find a *Herpetomonas* occurring naturally in the gut of the vertebrate. On one occasion, in the cloaca of two specimens of *Lacerata vivipara*, we found a uniflagellate monad, but it lacked a blepharoplast, and hence was not a *Herpetomonas*. Bayon (1915) states that he found a *Herpetomonad* in the cloaca of a *Chameleon pumilus* on Robben Island, while M. Léger (1918) states that he found a herpetomonad in the rectum of a lizard, *Anolis* sp. in Martinique. It is possible that these herpetomonads in the hind gut of lizards may have been acquired from ingested infected flies, and have passed thence into the blood of the lizards. It is also possible that in the catching of the fly on the tongue of the lizard, herpetomonads may have been liberated from the insect and have passed through the mucous membrane of the tongue of the vertebrate. On the other hand, they may have been inoculated into the blood of the lizard directly by the action of a bloodsucking fly or other Arthropod. We do not consider, on the evidence available, that herpetomonads are natural parasites of the gut of vertebrates, though they may be acquired from invertebrates by way of the gut and pass therefrom into other organs.

In our experience, when a herpetomonad is introduced into a vertebrate host, it may be able to exist either as a somewhat heavy infection that tends to die out and hence is transitory in the vertebrate, or it may become established in so attenuated a form that the pathogenic effects of its presence are not detected unless the resistance of the host is suddenly diminished. Again, owing to periodicity of multiplicative periods of the parasites, they may only be capable of detection in the blood of the host at certain seasons. The accidental, successful introduction of herpetomonad parasites by the agency of certain insects may thus afford an explanation of sporadic outbreaks of such diseases as kala-azar or other form of leishmaniasis.

When a herpetomonad gains access to and proves capable of multiplying in a vertebrate, though the infection may prove to be sparse, as in the case of *H. denticis*, it indicates that while there is still difficulty for the herpetomonad to live in the blood of the vertebrate, yet an attempt is being made that may become more successful—and perhaps more obvious—in the future. In other words, the presence of a natural herpetomonad in the blood and organs of *Dentex* indicates a further example of the habituation of a flagellate of invertebrates to life in a vertebrate host.

The herpetomonads, indeed, show wonderful powers of adaptation, one of the most plastic being *H. davidi*, natural to the gut of certain plant-frequenting insects, which is able to live in the latex of certain Euphorbiaceous plants.

Such diseases as leishmaniasis in vertebrates need not be regarded as necessarily being conveyed by any one specific insect carrier of herpetomonads, but as being transmitted more or less accidentally into a susceptible subject by the agency of any insect capable of being heavily parasited with herpetomonads, and of passing these flagellates into vertebrates.

The leishmaniasis are herpetomoniasis in which the dominant stage of the causal agent in the vertebrate is the rounded, resting, non-flagellate leishmaniform stage. The leishmaniasis are allied in causal agency, pathology and treatment (by tartar emetic) with the trypanosomiasis, wherein the dominant stage of the causal trypanosome in the vertebrate is the flagellate stage, but resting, non-flagellate, leishmaniform parasites also occur in the internal organs of the vertebrate.

The occurrence of natural herpetomonads in vertebrates, and the ability to infect vertebrates experimentally with herpetomonads—entailing pathogenic results resembling leishmaniasis in the case of warm-blooded hosts—present an interesting chapter in the evolution of disease.

SUMMARY

A new flagellate, *Herpetomonas denticis*, n. sp., occur naturally in the blood of fish, *Dentex argyrozona*, from St. James, near Cape Town. The parasite was also seen in spleen, liver and kidneys of the fish. Flagellate forms, 5 to 24 μ long, and 1.5 to 2.5 μ broad, occurred in the heart blood, and rounded, non-flagellate, leishmania-like forms were seen in the internal organs. Multiplication stages were found.

As far as known, this is the first record of the natural occurrence of a *Herpetomonas* in the blood and internal organs of fishes. Four *Dentex*, out of 41 examined, were scantily parasited. Herpetomonads were not found in the digestive tracts of the fish.

The significance of this piscine parasite is important, in view of the numerous experiments carried out by the authors and others on the successful infection of vertebrates with herpetomonads and their relation to *Leishmania*. The leishmaniasis are really herpetomoniasis of mammals, wherein herpetomonads—which are natural parasites of invertebrates, such as insects—have been introduced into vertebrates, such as mammals, with pathogenic effects.

REFERENCES CITED

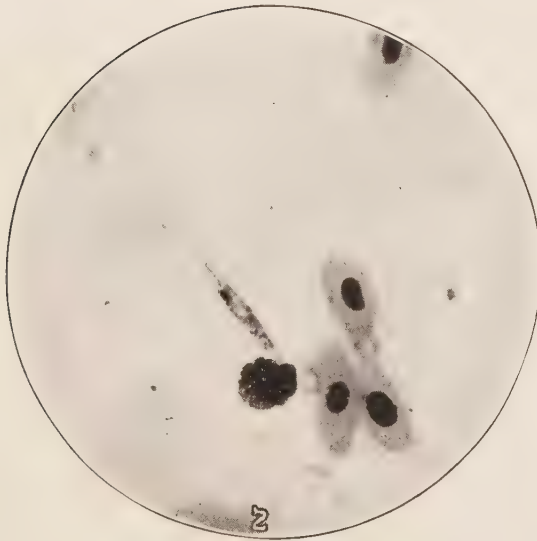
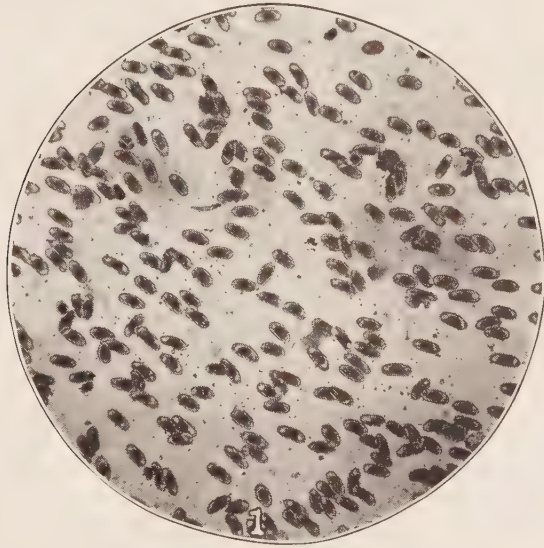
- Bütschli, O. 1884.—Protozoa. Brown's Kl. u. Ord. d. Tierreiches, vol. 3.
 Fantham, H. B., and Porter, A. 1914.—Some Insect Flagellates introduced into Vertebrates. Proc. Camb. Philosoph. Soc., 18:39-50.
 1915.—On the Natural Occurrence of Herpetomonads (Leptomonads) in Mice. Parasitol., 8:128-132.

- 1916.—The significance of Certain Natural Flagellates of Insects in the Evolution of Disease in Vertebrates. Journ. Parasitol., 2:149-166.
- Franchini, G. 1913.—Un nouveau protozoaire parasite de l'homme provenant du Brésil. Bull. soc. path. exot., 6:156-158.
- Laveran, A. 1917.—Leishmanioses. Paris. 521 pp.
- Léger, M. 1918.—Infection sanguine par *Leptomonas* chez un Saurien. C. R. soc. biol., 81:772-774
- 1919.—Hématozoaire flagellé nouveau dans une pyrexie cliniquement non classée. Bull. soc. path. exot., 12:80-84.

EXPLANATION OF PLATE III

Fig. 1.—Photomicrograph of the flagellate form of *Herpetomonas denticis* in the blood of *Dentex argyrozona*, obtained by using Zeiss 4 mm. apochromatic objective and Huyghenian 2 ocular

Fig. 2.—Photomicrograph of flagellate *H. denticis*, obtained by using Zeiss $\frac{1}{12}$ " oil immersion objective and Huyghenian 2 ocular.



THE DEVELOPMENT OF GREGARINES AND THEIR
RELATION TO THE HOST TISSUES: (III)
IN *GREGARINA RIGIDA* (HALL) ELLIS

MINNIE WATSON KAMM

This paper is the third of a series in which it is desired to show the effect of gregarines upon the host cells which they parasitize. In the two former papers the species considered were intracellular parasites, the young stages of which live within the intestinal cells and absorb their entire nourishment therefrom; they are consequently disastrous in their effect upon these cells. In *Cephaloidophora delphinia* (Watson) Kamm (1918) the minute trophozoite soon outgrows the parasitized cell and absorbs the walls of this and the adjoining cells, and finally comes to occupy considerable space within the epithelium, being highly deleterious in its action upon the host tissue. A moderate infection with this parasite therefore causes a serious gregarinosis in the host. It is questionable whether the host, the large white sand-flea *Talorchestia longicornis* (Say), is able to regenerate new tissue to replace the lost.

The first parasite studied (Kamm, 1917), *Stenophora lactaria* Watson, injures its host the milliped *Callipus lactarius* (Say), in much the same manner but to a less serious degree.

The present species, *Gregarina rigida* (Hall) Ellis, parasitizes species of the Acridiidae, the present specimens being taken from *Melanoplus differentialis* (Uhler). It is a particularly good species for sectioning because the percentage of infection runs high and the number of parasites per host is large.

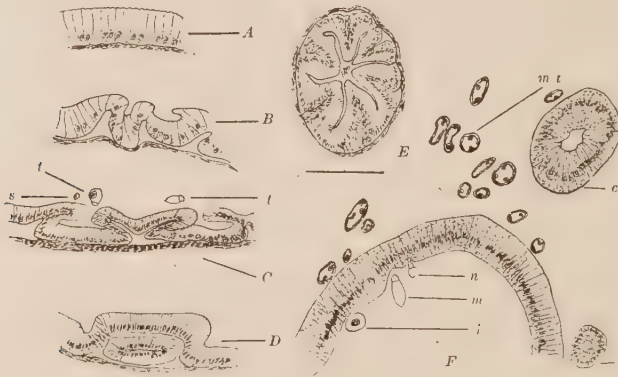
The entire alimentary tract (Fig. 1) was sectioned in order to determine the histology of the cells in the various regions and the degree of parasitism involved, this degree being dependent upon the character of the cells. The walls of the crop are chitinous and armed with teeth and are therefore not adapted to parasitism. The thin-walled, highly vascular mid-intestine is, on the contrary, well adapted to the existence of gregarines; I have found as many as fifty-four parasites in one cross-section made through the anterior portion of this region.

The six pyloric ceca originate near the anterior end of the mid-intestine (Text figs. A-F) and pass both forward and back, the backward-directed portions being only about one-third as long and much more slender than those directed anteriorly. They are admirably adapted to the early requirements of the parasite. The tiny sporozoites find lodging here without danger of currents of food or strong mus-

cular contractions tearing them loose from their hold on the cells. Some unilocular stages are to be encountered in the mid-intestine walls but by far the larger number is situated here. Consequently the larger epimerited trophozoites and smaller sporonts are abundant here also.

The Malpighian tubules which open into the alimentary tract at the junction of mid-intestine and intestine proper are not parasitized with gregarines.

The anterior end of the intestine proper is similar histologically to the mid-intestine and contains some gregarines but the rectum, which is chitinous-walled, is free.



Text Figure A-F.—*A*, *B*, *C*, and *D* represent the origin of the ceca at the junction of crop and mid-intestine. *A*, cell wall near posterior end of crop. *B*, convolutions in wall. *C*, double folds in wall, cells small, several gregarines near folds, *t* small sporonts, *s* sporozoite, moving freely from *c* ceca to intestine. *D*, cecum closed off from intestinal epithelium but still within longitudinal muscular layer. It soon penetrates this wall and lies freely in the coelom. *E*, cross-section through pyloric orifice of host between crop and mid-intestine. *F*, cross section through anterior end of mid-intestine and through the small backwardly-directed ceca (*c*) and the anterior end of the Malpighian tubules (*mt*); one trophozoite (*n*) is attached to the cell-wall, one small sporont (*m*) is cut longitudinally and a larger one (*i*) crosswise.

All drawings were made at same magnification, the line (under *E*) representing 200μ .

Sections were cut thin (5μ) so as to include portions of the same young parasite in several successive sections. Stains used included Delafield's and iron hematoxylin counterstained with orange G; best results were obtained with the early stages by using the latter but for the larger parasites the former was the more satisfactory. In order to determine the effect of the parasite upon cells, both stains were useful, the iron stain for the chromatin and Delafield's for cytoplasmic modifications, if any.

Free sporozoites (Fig. 2) were found in large numbers in both mid-intestine and ceca. Much to my surprise they are spherical or sub-spherical in shape, measuring 14 to 20 μ in diameter, the nucleus being proportionately large and filled with scattered granules of chromatin.

Upon coming in contact with the epithelium, there is pushed out from the sporozoite a small conical protuberance (Fig. 3) at first devoid of endoplasm. This papilla elongates into a slender neck, often as long as the sporozoite itself, and forces itself through the intima into the wall of the intestine. This spherical sporozoite and its ameboid character have not to my knowledge been heretofore reported for gregarines but from many hundreds of observations with high power, I am convinced that these phenomena exist. The protuberance has been seen in its incipency through the gradually growing stage when the sporozoite lies adjacent to the wall and to the penetration of the long, now pyriform, trophozoite which becomes firmly attached.

In none of the observations made was the cell-wall punctured, the sporozoite pushing up between two cells and splitting them apart instead. This is obviously the easier process for the apex is unarmed and yet it affords a holdfast, which is all the young parasite desires. The point often enters the cell area at considerable slant and the parasite is unable to recover its normal perpendicular position, possessing a crooked epimerite during the rest of its trophozoitic life.

Sporozoites are generally found in the recesses between folds of the ceca where they collect in groups and are fully protected. They also sink into recesses which appear to have been made by parasites which have left their holdfasts and become free-living sporonts. The more exposed regions are more infrequently parasitized because the sporozoites which have endeavored to attach themselves here have been swept away.

Very many faintly-staining bodies the size and shape of sporozoites are often found massed along the periphery of the lumen, their nuclei generally disintegrated and their outline often indefinite. I venture the suggestion that these are dead or infertile sporozoites; and that sporozoites must be eaten by the proper host within a certain time, probably a few weeks after extrusion from the cyst, in order to remain fertile. The similarity in size and shape preclude the possibility of their being sporozoites of other gregarines not infective in this particular host.

When the process of the sporozoite is first thrust into the cell-area it is ectoplasmic but it soon fills with endoplasm and swells into a knob-like holdfast not easily dislodged (Fig. 5). A septum begins

to develop although not at first visible but evident from the differential staining. The trophozoite has now fully established itself and is growing rapidly. It has crowded several cells aside at their apices, not only the epimerite but a goodly portion of the protomerite being pushed up into this cell-area.

An instance in which the entire trophozoite is pushed up into the cell area is described by Léger and Duboscq (1899) for *Gregarina davini* Léger and Duboscq. The parasite has located in a recess between two lobes, its large dilated epimerite occupying the regenerative space at the base of the epithelium, which contains many nuclei but no cell walls. It transforms this space into "un kyste épithélial." The nuclei are massed near the epimerite but are not in the least affected by its presence.

The septum is now established and the staining in the two parts that are characteristic of the sporont; the nucleus is very large and contains many karyosomes which later fuse into one; and the epimerite now contains a small amount of endoplasm only near its base.

If the epithelium were being affected by the parasite this would now be evident. The cells are undoubtedly crowded and their nuclei often pushed out of place and shape; but upon careful observations with both stains I can detect no difference in character between the normal and crowded cytoplasm or between the normal and distorted nuclei. There is no hypertrophy and I do not think the crowding could be called atrophy for the cells return almost to their normal shape. The larger the parasite becomes the less its presence disturbs the cells, for the body retreats into the lumen except for the small epimerite (Figs. 6, 7). I think the cell always retains a trace of the indentation made by the epimerite and that this depression is taken advantage of by the young sporozoites as a new holdfast.

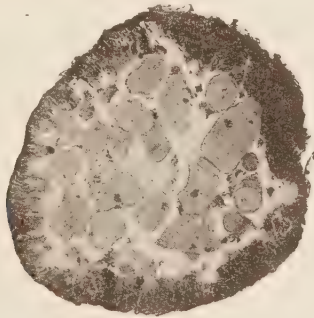
The epimerite is gradually constricted as the parasite grows, until it becomes a perfect sphere, completely pinched off, leaving a small indentation but no open wound in the apex of the protomerite (Fig. 9) which soon smooths out. Vigorous muscular contractions of the intestine or the current of food carries the sporont away and leaves the epimerite in the cells (Fig. 10), where it soon atrophies and disappears.

If its movements are too strenuous or outside mechanical means interfere, the parasite may lose its epimerite prematurely (Fig. 11). I doubt if it is able to recover the loss; it is probable that a thin stream of protoplasm continues to flow from the apex until the protomerite collapses and the animal dies. The parasite is probably unable to regenerate a new epimerite and a new fully developed organ would be unable to penetrate the epithelial cells.

Large free sporonts in the ceca are the exception rather than the rule. A maximum length of 90μ was observed here, the animals soon moving out into the intestine, where more food is to be found. This migration is not due to any new chemical affinity which the parasite acquires, for the weak acid secreted by the gastric ceca passes directly into the mid-intestine, where the parasites continue to be bathed in it; the migration is unexplainable. The fact that the ceca become too small is not adequate explanation.

In the intestine the sporonts are tightly compressed between food-masses the greater part of the time, since the host eats frequently, and they are often flattened so that in cross-section one dimension is three times the other. The parasites just as readily adhere to food masses as to the intestinal walls, which means that they are frequently carried to the exterior in food-pellets.

Two sporonts in association preparatory to the formation of a cyst are shown in the microphotograph (Text fig. G).



Text Figure G.—Microphotograph of section through mid-intestine showing lumen almost completely filled with parasites cut at various angles, the short wavy outlines denoting movements when fixed. Four associations of adult sporonts are shown.

In comparison with the number of parasites encountered, the number of cysts is very small, so the gregarines which are able to complete their life-history are comparatively few in number.

One more paper will be presented in this series, on another epimerited species of a genus not closely related to the genus *Gregarina* and a bibliography will be given covering work on Effect on Host Tissues.

SUMMARY

1. Ten successive stages in the life-history of *Gregarina rigida* (Hall) Ellis are shown from the spherical sporozoite free in the lumen of the intestinal tract to the associative sporonts.

2. This species possesses an epimerite which develops in the sporozoite as an ameboid papilla, becoming a long slender neck, which is thrust through the intima between two epithelial cells rather than into one, where it obtains a holdfast and develops at the cell-apex a rounded knob for an epimerite.

3. The cells parasitized are affected only mechanically, being pushed aside during the parasite occupancy; no chemical effect upon the cell is noted at any stage of occupancy and the cell is apparently uninjured.

BIBLIOGRAPHY

- Kamm, Minnie Watson. 1917.—The Development of Gregarines and Their Relation to the Host Tissues: (I) in *Stenophora lactaria* Watson. Jour. Parasit., 3:124-30.
 1918.—The Development of Gregarines and Their Relation to the Host Tissues: (II) in *Cephaloidophora delphinia* (Watson). Jour. Parasit., 5:35-40.
 Léger, L., and O. Duboscq. 1899. Notes biologiques sur les Grillons. C. R. Soc. Biol., (3) 7:xxxv-xl

EXPLANATION OF PLATES

All drawings except Fig. 1 were made with camera lucida from sections cut at 5μ . The line in drawings 2, 3, 4, and 5 represents 10μ , in the other drawings 100μ .

EXPLANATION OF PLATE IV

Fig. 1. Alimentary tract of *Melanoplus differentialis* (Uhler), after Folsom.

<i>p</i> pharynx	<i>m</i> stomach or mid-intestine
<i>o</i> esophagus	<i>mt</i> Malpighan tubules
<i>gl</i> salivary glands	<i>i</i> intestine
<i>a</i> crop	<i>j</i> colon
<i>g</i> gastric ceca	<i>r</i> rectum

Fig. 2.—Two sporozoites free in cecum.

Fig. 3.—Sporozoite with apical protuberance developed ready for penetration into cell area. See also Fig. 13.

Fig. 4.—Portion of cecum with three sporozoites in the process of entering the cellular area, two sharply pointed and one with blunter apex. One sporozoite with elongated protuberance lies near the epithelium. See also Figs. 12 and 13.

Fig. 5.—Slightly knobbed epimerite in young trophozoite in which a difference in staining reaction is discernible between protomerite and deutomerite.

Fig. 6.—A large trophozoite crowded between two lobes of the cecum.

Fig. 7.—A large trophozoite in epithelium with its epimerite somewhat eccentric.

Fig. 8.—Trophozoite with epimerite and septum developed. The epimerite is held in place by pressure from the outside rather than by a specialized holdfast.

Fig. 9.—Sporont with indentation at apex where epimerite has recently become detached.

Fig. 10.—Sporont which has just become constricted from its epimerite.

Fig. 11.—Trophozoite which has become prematurely severed from its epimerite.

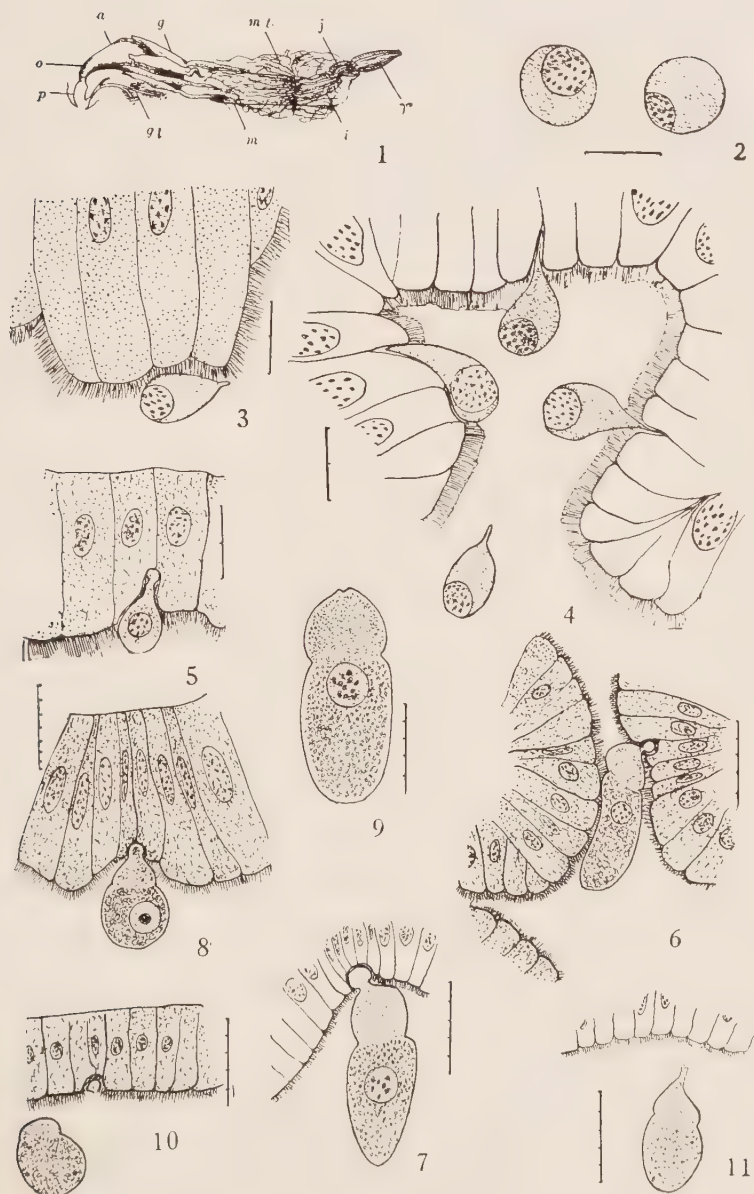
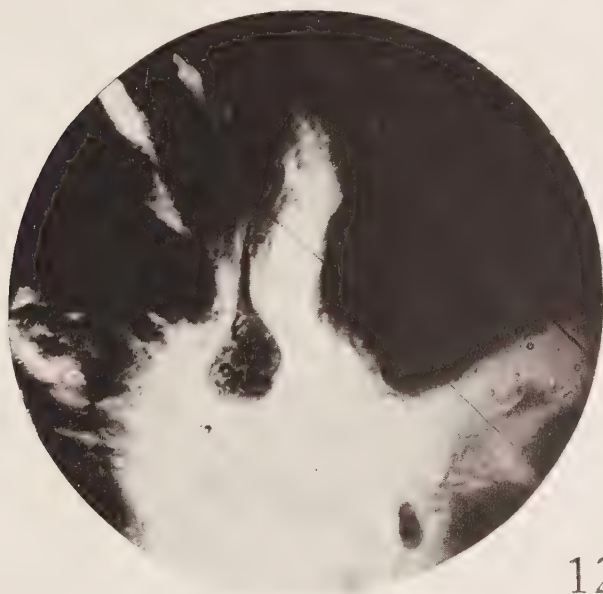


PLATE IV

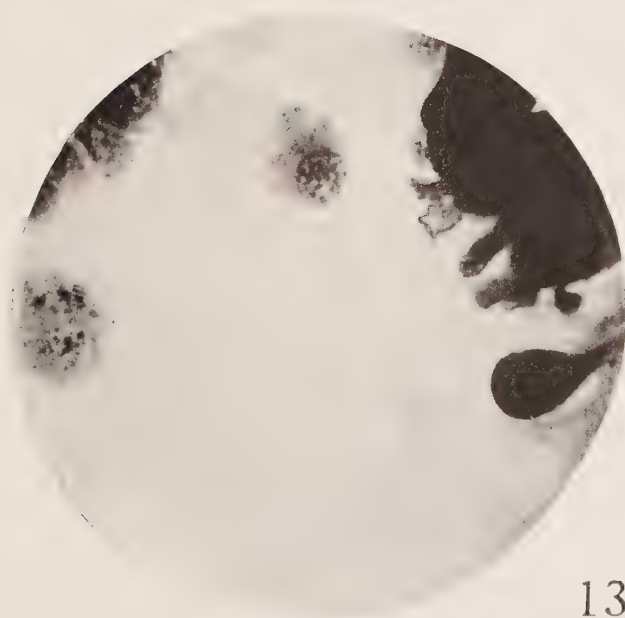
EXPLANATION OF PLATE V

Fig. 12.—Microphotograph (\times about 1200) showing pyriform sporozoite about to penetrate into area between cells. Note large nucleus upon face of cell.

Fig. 13.—Microphotograph (\times about 1200) showing three sporozoites, one at right, with protuberance half developed, lying near epithelium; other two with apical ends at lower focus than body of cell but showing the rotund character of the sporozoite at its distal portion, and the large nucleus which contains many distributed karyosomes.



12



13

A NEW NEMATODE FROM THE RAT*

SADAMU YOKOGAWA

FORMOSA MEDICAL COLLEGE

During the winter of 1920, while I was working at Johns Hopkins University, Dr. W. W. Cort assigned me for investigation the problem of the nematodes of rats and gave me some nematodes, which he had collected from a Norwegian rat, *Epimys norvegicus*, caught in Baltimore. These worms were found to be a new species of *Heligmosomum*, to which I give the name *Heligmosomum muris* nov. spec. Later I found this same species in twenty-four out of twenty-six rats examined, which were caught near Baltimore. Other nematodes found were *Strongyloides papillosus*, *Trichosomoides crassicauda*, *Hepaticola hepatica* and *Heterakis spumosa*, but the present paper describes only *Heligmosomum muris*, since the others have been described by other authors.

Although *Heligmosomum muris* is a common parasite of rats here, nobody seems to have found it previously. My studies on the structure of this species were made for the most part from living material, although specimens preserved in alcohol were used for comparison. The measurements of the body were made on fixed material, because the size of the living worms is changed by movement. Accordingly the sizes given in this paper are a little smaller than those of the living worms.

Specimens of *Heligmosomum muris* preserved in alcohol are dark brown in color, filiform and coiled irregularly two to five times. These worms live in the upper part of the small intestine, especially near the duodenum of the rats. When still in position in the intestine, the worm appears as a little curved red string in the mucus or buried slightly in the mucous membrane. If, however, cool normal saline is poured on the intestine or the rat is not dissected soon after it has been killed, the worms will always be found more or less coiled. In the living worm, the body is red, filiform, and somewhat narrowed anteriorly. The head is small, 21 to 25 μ in diameter exclusive of the surrounding cuticular expansion, and 30 to 36 μ in diameter including it (Fig. 1). The mouth is small and a small oral cavity (*a*) is present. In an optical section of this region in worms preserved in alcohol the sub-cuticular part of the circumoral area gives somewhat the appearance of two small teeth, one on each side. The esophagus is conoidiform, a little sinous and 0.35 to 0.45 mm. long. The cuticula has transverse striations and prominent longitudinal markings in the form

* This paper is a contribution from the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

of ten ridges. It is relatively thick and inflated on the head and the anterior cervical region, making a cephalic area or expansion. The length of this area is 0.06 to 0.07 mm. The longitudinal cuticular ridges originate a little behind the cephalic area and run parallel clear to the posterior end. The transverse striations are not continuous around the cuticula but are found only on the longitudinal ridges. The excretory system is well developed and takes the form of two elongate sacs, which contain a large amount of highly refractile granules. These sacs are situated on each side of the body and extend to about the middle of the body. The excretory sacs are connected with the excretory pore by a small canal. The excretory pore is situated on the ventral surface, at a distance of 0.8 to 0.14 mm. in front of the base of the esophagus. The nerve ring is situated just in front of the excretory pore at a distance of 0.2 to 0.25 mm. from the anterior end of the esophagus. Cervical papillae are not present. The cells lining the intestine contain a large amount of melanin-like pigment. This is distributed throughout the entire length of the intestine.

The male of *Heligmosomum muris* (Fig. 2) is smaller than the female, being 3 to 4 mm. in length with a maximum thickness of 0.085 to 0.1 mm. at the middle of the body. The bursa (Fig. 3) is large and a little enroled, expanding toward the ventral side. It consists of two large lateral lobes and a small dorsal lobe. The lateral lobes are each supported by six rays and are asymmetrical, the right being larger than the left. In one worm measured, the right lobe was 0.218 mm. long and 0.164 mm. wide, and the left lobe was 0.164 mm. long and 0.146 mm. wide. The rays of the left lobe are more divergent than those of the right, and are different in form on the two lobes. The ventro-ventral, latero-ventral, externo-lateral and medio-lateral rays of the left lateral lobe are similar in form and have almost equal intervals between them. The left postero-lateral ray has a very different form, being shorter and wider than the others and diverges from the medio-lateral ray, curving posteriad. The postero-lateral ray of the right lobe is relatively small and diverges from the medio-lateral ray, curving a little posteriad. The medio-lateral and externo-lateral rays of the right lobe are digital and larger than the other rays. They run close together and parallel throughout most of their extent, but their tips diverge. The latero-ventral ray of the right lobe is long and straight and the right ventro-ventral ray is slender and quite divergent. Both externo-dorsal rays (*ed*) are thin and slender. They diverge from the root of the dorsal ray and run along the boundary line between the two lateral and the dorsal lobes, curving posteriad; consequently they sometimes appear to belong to the lateral lobe and sometimes to the dorsal. The dorsal lobe is very small and divided from the lateral lobes by

shallow indentations. The dorsal ray (*d*) is 45 to 54 μ long and terminates in four digitations.

The body of the male terminates posteriorly in a thin cone which projects into the bursa along the anterior surface of the dorsal lobe. The two spicules are yellowish brown, about 0.56 mm. long and filiform. They are united at their distal ends and form a small arc. The two gubernacula are colorless and situated on the ventral and the dorsal sides of the distal end of the spicules. The ventral gubernaculum is longer than the dorsal. The former is 0.06 to 0.07 mm. long and the latter 0.04 to 0.05 mm. long.

The anterior part of the testis (Fig. 2, *c*) is situated on the dorsal side near the beginning of the intestine and is loop-shaped. Its beginning is very difficult to see because it is covered with the large excretory sac. However, I could see it sometimes clearly in the living worms, and found that the distance of the anterior tip of the testis from the base of the esophagus varied somewhat in different worms. At the middle of the body, a narrow region (*d*) 0.02 to 0.03 mm. long follows the testis. The wall of this region is thick and consists of cuboidal cells, similar to the cells of the ejaculatory duct. It seems to be a vas deferens because it is joined immediately to the seminal vesicle (*e*) filled with spermatozoa. The seminal vesicle is 0.1 to 0.13 mm. long and is followed by a narrow canal 0.09 to 0.12 mm. long which is surrounded by high columnar cells (*f*). Each of these cells contains a round nucleus and many granules. The cells which are situated on the posterior half of this canal are darker than those of the anterior half. These cells probably correspond to the cement glands of other forms, and the darker coloring appears to be connected with the secretory activity of the cells. This part connects with a well developed ejaculatory duct. The wall of the ejaculatory duct (*g*) consists of a layer of transparent cuboidal cells. At the level of the cement gland the reproductive tube crosses the intestine again. Consequently the anterior half of the intestine is located on the ventral side and its posterior half on the dorsal side of the reproductive tube. The distal end of the intestine joins the posterior end of the ejaculatory duct.

The female (Fig. 4) of *Heligmosomum muris* is larger than the male. It is 4 to 6 mm. long, with a maximum thickness of 0.09 to 0.12 mm. in the middle of the body. In the contracted worm the posterior region of the body is withdrawn to such an extent that the cuticula forms a sac surrounding the anus and vulva. The posterior end of the body becomes reduced suddenly in size just behind the vulva, terminating in a short, thin tail. The anterior end of the ovary (*h*) bends and forms a small loop. This part is situated on the dorsal side of the anterior part of the intestine at a varying distance from the base of the esophagus. The blind free anterior

end of the ovary contains extremely small cells, the primordial germ cells. The main part of the reproductive organ is situated on the dorsal side of the body and the ovary is filled with developing oocytes, which generally arrange themselves in single file. The ovary connects with the receptaculum seminalis by a narrow tube near the posterior part of the body. The receptaculum seminalis (*i*) is situated at the beginning of the uterus without sharp demarcation. The uterus is situated in the posterior part of the body and is 0.45 to 0.60 mm. long, containing 13 to 27 eggs, and connects with the ovejector (*j*) of about 0.1 mm. in length. The ovejector is joined to the thick walled vagina, crossing the distal end of the intestine. The ovejector has a well developed wall which at the beginning of the ovejector is thickened forming a sphincter. The ovejector seems to be a little twisted and its distal end projects into the vagina. The vagina (*k*) is 0.14 to 0.16 mm. long and situated on the ventral side of the body. Its wall is lined by cuticula. There are strong irregular longitudinal folds on its internal surface and it extends into the distal end of the ovejector. The vagina runs in a diagonal direction in the posterior end of the body, and terminates in the vulva. This (*l*) is situated on the ventral surface just in front of the anus at a distance of 0.1 to 0.13 mm. from the tip of the tail. The intestine is straight and runs along the ventral side of the body, terminating in the anus. It crosses the ovejector near the posterior end of the body. The anus (*m*) is situated at a distance of about 0.06 mm. from the tip of the tail. The eggs are ellipsoidal with a very thin shell. The average size is 58μ by 33μ ; a common minimum is 54.6 by 30.9μ and a frequent maximum 61.8 by 34.5μ . They are in the one to sixteen cell stages of development in the uterus and in the feces are in the four to sixteen cell stage, exceptionally, also in the morula stage.

In the genus *Heligmosomum*, Railliet and Henry the following species have been placed: *Heligmosomum costellatum* (Dujardin), *H. minutum* (Dujardin), *H. gracile* (F. S. Leuckart), *H. laeve* (Dujardin), *H. braziliense* Travassos, *H. agoutii* Neiva, da Cunha and Travassos, *H. vexillatum* Hall and *H. cristatum* Geddoelst. All of these are discussed by Hall (1916: 149-158) except *H. agoutii* Neiva, da Cunha and Travassos (1914) and *H. cristatum* Geddoelst (1917). Seurat (1915) gives a detailed description of *H. laeve* Dujardin, which is not fully covered in Hall's paper. *Heligmosomum muris* is most closely related to *Heligmosomum vexillatum* Hall, which was described from *Thomomys fossor* from Colorado, and to *Heligmosomum brasiliense* Travassos from the Norwegian rat from Rio de Janeiro, Brazil. A comparison of *H. muris* with the description of *H. vexillatum* and with a toto mount of this species which was kindly loaned me by Doctor Hall, showed that while these two species agree in general size and shape, they are strikingly

YOKOGAWA—NEW NEMATODE FROM THE RAT

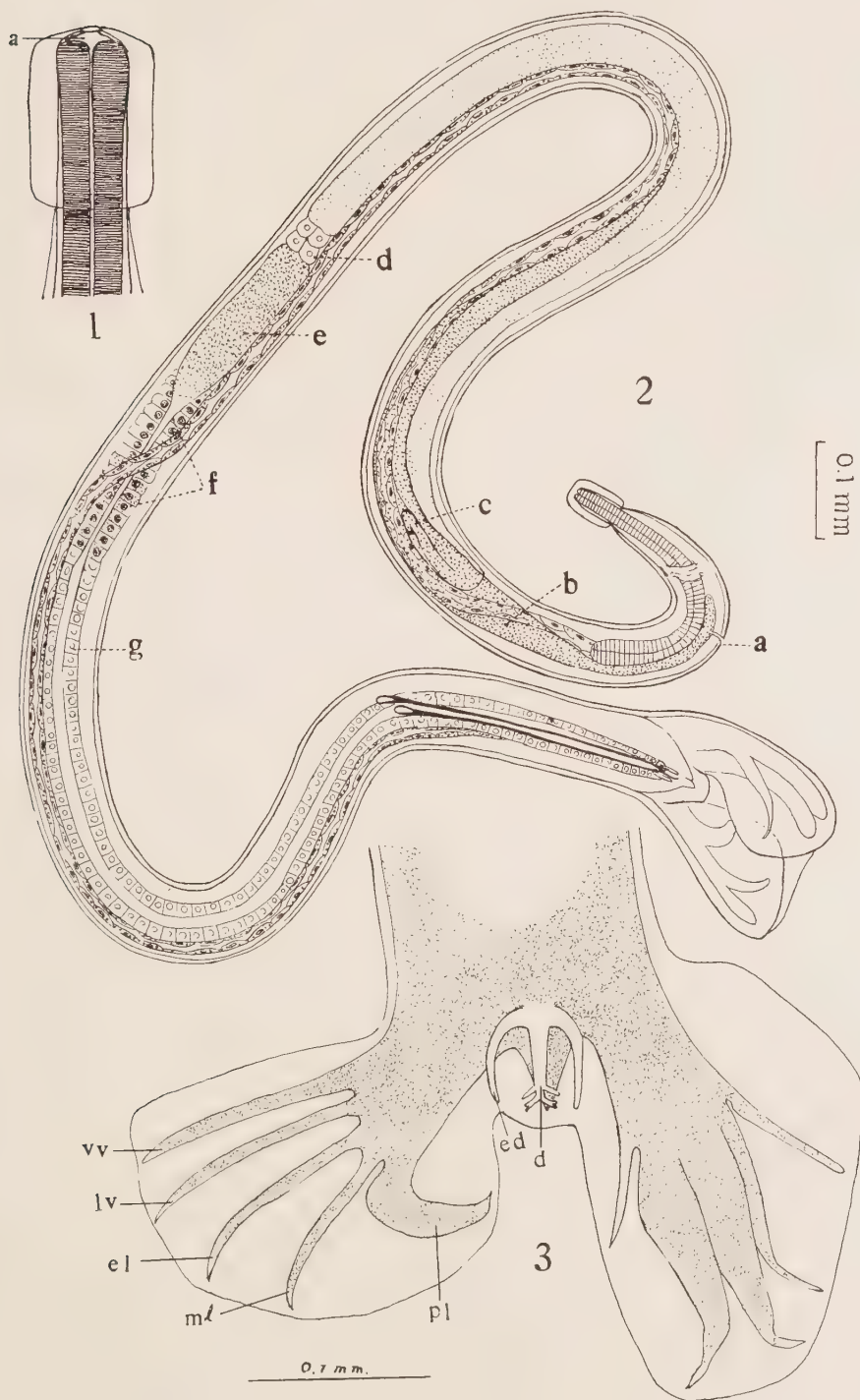
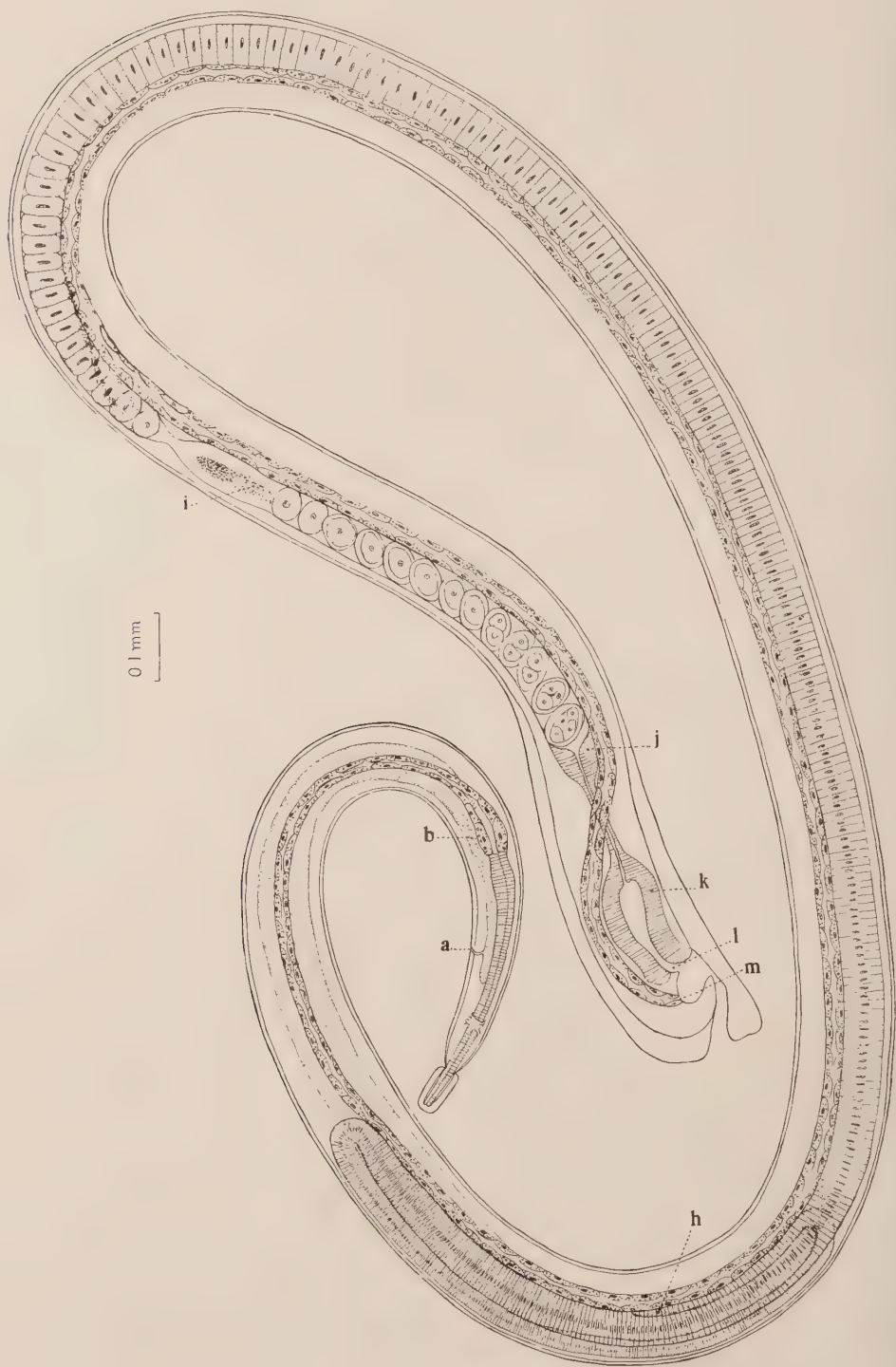


PLATE VI



different in the length of the spicules, the size of the eggs, and the character of the bursae. A comparison of my species with *H. brazil-iense* is more difficult since this species is not figured and not described in detail, especially in regard to the character of the bursa. Differences are apparent in the size of the eggs, the length of the esophagus, the structure of the bursa, and the position of the anus and vulva. The most striking differences are in the size and shape of the two species. In *H. brazilense* the male is from 2.6 to 2.8 mm. in length and 0.09 to 0.1 mm. in diameter at its widest part, giving the ratio of length to width of about 30:1. The female is 3.5 mm. long and 0.13 mm. in width with a ratio of length to width of about 27:1. *H. muris* is longer and especially in the female much narrower for its length, as in this species the male has a length of 3 to 4 mm. and a width of 0.85 to 0.1 mm., making a ratio of length to width of about 33 to 44:1, and the female has a length of 4 to 6 mm. and width of 0.09 to 0.12 mm., making a ratio of length to width of 44 to 50:1. These differences seem to me to make it necessary to establish *Heligmosomum muris* as a distinct species.

I wish to express here my deep indebtedness to Doctor W. W. Cort, under whose direction this work was carried on, and also to Doctor B. H. Ransom and Doctor M. C. Hall for their kindness in helping me with the preparation of this paper.

PAPERS CITED

- Geddoelst, L. 1917.—Nematodes parasites du *Sciurus prevosti* de Sumatra. Rev. zool. Africaine, 5: 153-162.
 Hall, M. C. 1916.—Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea. Proc. U. S. Nat. Mus., 50: 1-258.
 Neiva A., da Cunha, A. M., and Travassos, L. 1914.—Parasitologische Beitrage. Memorias do Instituto Oswaldo Cruz, 6: 180-191.
 Seurat, L. G. 1915.—Sur les conditions de la ponte du strongle lisse. Bull. scient. France et Belg., 48: 171-177.
 Travassos, L. 1914.—Tricostrongylidoes brasileiros. Brazil medico, 28: 325-327.

EXPLANATION OF PLATE VI

Fig. 1.—The anterior end of *Heligmosomum muris*, shown in optical section; *a*, mouth cavity.

Fig. 2.—Male of *Heligmosomum muris* from an experimental rat, 9 days after infection, latero-ventral view; *a*, excretory pore; *b*, excretory sac; *c*, anterior end of the testis; *d*, vas deferens; *e*, seminal vesicle; *f*, cement gland; *g*, ejaculatory duct.

Fig. 3.—Bursa of male of *Heligmosomum muris*, dorsal view; *d*, dorsal ray; *ed*, externo-dorsal ray; *pl*, postero-lateral ray; *ml*, medio-lateral ray; *el*, externo-lateral ray; *lv*, latero-ventral ray; *vv*, ventro-ventral ray

EXPLANATION OF PLATE VII

Fig. 4.—Female of *Heligmosomum muris* from the wild rat. *a*, excretory pore; *b*, excretory sac; *h*, anterior end of ovary; *i*, seminal receptacle; *j*, ovejector; *k*, vagina; *l*, vulva; *m*, anus.

A NEW RECORD OF *TAENIA CONFUSA*, WITH
ADDITIONAL NOTES ON ITS MORPHOLOGY*

ASA C. CHANDLER

In a collection of parasitological specimens which the writer recently received from Dr. Mark F. Boyd of the Department of Bacteriology and Preventive Medicine at the University of Texas Medical School, Galveston, there was a specimen of a tapeworm which upon investigation was found to be, apparently, an example of *Taenia confusa* Ward 1895. The worm had been sent to Dr. Boyd from the medical school hospital as a specimen of *Taenia saginata*, and was given to the writer as such, without ever having been more than casually examined. Efforts are at present being made by Dr. Boyd to get some information as to the origin of the worm, but to this date these efforts have not been successful. Nothing definite can be said at present as to its origin except that the patient was probably a native of Texas.

Particular interest attaches to the occurrence of this worm, inasmuch as hitherto only two specimens of *Taenia confusa* have been recorded, both having been sent, at different times, to Dr. H. B. Ward by a physician at Lincoln, Nebraska, in 1895. During the twenty-five intervening years no further specimens have been discovered, yet the present occurrence of a specimen from an individual in Texas indicates a strong probability that the worm has existed throughout this time in the Southern part of the middle western portion of the United States in sufficient numbers to protect it against extermination. It is probable that, as in this case, it may frequently have been passed over as a specimen of *Taenia saginata*.

Although in general agreeing with the description of *Taenia confusa* as given by Guyer (1898), the present worm differs in some details, especially of measurements, though not to such an extent as to throw serious doubts on its identity. It is, however, important to note that this worm largely bridges the gap between *T. confusa* Ward 1895 and *T. bremneri* Stephens 1909. The description of the latter is very meager, but the principal difference between this species and *Taenia confusa*, as far as determined by the few segments from which Stephens wrote his description, is in the greater width of the terminal segments, and in the greater abundance and larger size of the calcareous bodies. In both these respects the present worm is

* Contribution from the Biological Laboratory, Rice Institute, Houston, Tex..

intermediate between *T. bremneri* and *T. confusa*. According to Dr. Bremner, who sent the specimen to Stephens from northern Nigeria, "All Fullani (a Nigerian tribe) women have them, and they are got thru drinking sour milk." Since many of the American negroes originally came from Nigeria, the occurrence of this worm in the Southern United States would very readily be explained. It is, therefore, proposed that until further evidence to the contrary is obtained, *Taenia bremneri* be considered a synonym of *Taenia confusa*.

Before discussing any of the details of the present worm, a brief account of the general morphology and anatomical peculiarities of *Taenia confusa* as described by Guyer (1898) is in place. *T. confusa* is a tapeworm from 5 to 8 meters in length, consisting of from 700 to 800 proglottids, almost all of which are longer than wide. The terminal proglottids are from 27 to 35 mm. long by 3.5 to 5 mm. wide. The scolex is not certainly known. One of Ward's specimens was provided with a scolex and Ward (1897) states that this scolex was studied by him, still attached to the entire chain, under a lens, and that it was approximately the size and shape of the scolex of a *Dipylidium*. This head was cut off, stained, and mounted by an assistant. It proved to be so much like the head of a *Dipylidium* that Dr. C. W. Stiles, according to Ward, stated that it could be nothing else. Ward states that so far as he is aware there was no opportunity for it to be confused with the head of another tapeworm, but on the evidence of the improbability of a *Taenia* having a head so strikingly like a *Dipylidium*, he was unwilling to record the head as that of the worm he was studying.

The principal anatomical features of the worm, as described by Guyer, which differentiate it from other human *Taeniae* are the following: delicate cuticle and musculature; small sparse calcareous bodies; small testes; small shallow genital pore, with plug-like papilla nearly filling it; vagina with distinct receptaculum seminis, preceded by a short, constricted, thick-walled portion, and with cilia doubtful, and if present pointing towards the pore instead of away from it; shell gland oval, traversed by vaginal canal, and connected with uterus by separate egg canal opening into dorsal side of uterus; ovaries large, kidney shaped; vittellaria triangular, unpaired, wedging in between ovarian lobes; ripe uterus with median stem and 14 to 18 irregularly disposed and irregularly ramifying branches, with a series of finger-like branches transversely arranged across the anterior end, the eggs emptying out before disintegration of the segment; eggs oval, $30\mu \times 39\mu$, without evident pyriform apparatus.

The general morphology of the present worm agrees in most details with that of Ward's specimens as described by Guyer, the scolex not being considered. The worm here described consists of

approximately 790 segments, the great majority of which are longer than wide. The terminal segments of this worm, measuring from 25 to 33 mm. in length, are from 6 to 8 mm. in width, as compared with a width of from 3.5 to 5 mm. in Ward's specimens, and of 9 mm. in *Taenia bremneri*. The width of the segments which are past sexual maturity but not yet fully ripe increases to about 9 mm., this width being retained for a long distance in segments gradually increasing in length from 9 to 20 mm. The approximately square segments measuring 9 by 9 to 10 mm. agree with Guyer's measurements for segments 150 to 250 cm. back of the head, which are 10 mm. long by 9 to 10 mm. wide. The difference in the width of the terminal segments may very possibly be due to a difference in the state of contraction, especially inasmuch as the worm here described does not have such conspicuously flaring posterior ends on the proglottids.

There are a number of differences between this worm and Ward's specimens in the measurements of organs. Some of the larger measurements of the present worm may be partially accounted for by the fact that the measurements are for sexually mature proglottids which measure about 9 by 9 mm. and in which the uterus is already provided with branches, whereas Guyer's measurements appear to be for the organs as they appear in much smaller segments, with unbranched uterus, which he considered sexually mature, possibly relying too much on analogy with *Taenia saginata* or *Taenia solium*. The genital pore measures from 0.8 to 1.2 mm. across by 0.25 mm. in depth, thus resembling *Taenia saginata* much more closely than do Ward's specimens. The structure of the genital pore region is similar as regards the plug-like papilla which nearly fills it, and at the tip of which the cirrus opens. It differs, however, in that the vagina also opens at the tip of the plug, just posterior to the opening of the cirrus; in fact, there is a very short common opening, about 50 μ in depth.

The vagina has the peculiar features described by Guyer. In this specimen the cilia are very distinct and, as suspected by Guyer, point *towards* the genital opening, instead of away from it. Just before entering the lens-shaped receptaculum seminis there is an abrupt reduction in the lumen of the vagina with a much increased thickness of the walls, as described by Guyer. It has not been possible in the new worm to trace out the egg duct from shell gland to the dorsal wall of the uterus, but the uterus does not appear to enter the shell gland directly. The vas deferens is as described by Guyer, much coiled, and ends near the middle of the segment. In a few mounted proglottids the vasa efferentia leaving the vas deferens show very clearly, particularly so in a proglottid represented in figure 1. The ovaries in proglottids in which the uterus is unbranched are

about 1.8 by 0.65 mm. and 1.3 by 0.6 mm. respectively (the segment measures 6.5 by 4.5 mm.) but the segments do not have the reproductive organs of either sex fully matured until they reach a size of approximately 9 by 9 mm., and have the uterine branches already evident. In such segments the larger of the fully developed ovaries measures 2.7 by 1.5 mm. The vitellaria vary considerably from the broad and narrow form shown in figure 1 to a short and wide triangular form as figured by Guyer; the scalloped posterior edge is a constant feature. The ripe uterus is as described by Guyer; the most salient feature is the great irregularity of the short deeply subdivided branches which frequently become constricted at the point of emergence from the main stem; the terminal twigs, on the other hand, are swollen and contiguous. There is a series of forward-projecting finger-like branches at the anterior end, and there are two or three deeply-cleft branches prolonged in a backward direction, the main stem of the uterus not extending back of the shell gland. The type of branching of the ripe uterus is reminiscent of that of *Taenia hydatigena* Pallas. The testes in the present worm measure from 105 to 125 μ in diameter, as compared with 89 to 96 μ according to Guyer, and 150 μ in *Taenia saginata*. A few testes near the junction of the vasa efferentia with the vas deferens are greatly enlarged, and may be 195 μ in diameter, as shown in figure 1.

The uterine eggs of the present worm are approximately the size and shape reported by Guyer for Ward's specimens, though the majority are a little larger (33 by 42 μ). There is a distinct pyriform apparatus in the form of two short filaments attached to the thin outer shell as shown in figure 2.

The scolex is the most interesting part of the worm here described. Although the scolex was attached to only a small portion of the body of the worm, there seems to be no reason for doubting that the head really belongs to the worm here described. There are no segments of any other worm associated with this one to indicate a double infection, and the breadth of the neck attached to the head is the same as that of the smallest section of the proglottids. Moreover, the head, although unquestionably a Taeniid head, is quite different from that of any other human species of tapeworm. It does not in any way resemble the head of *Dipylidium*.

The scolex is unarmed, and is very sharply demarcated from the neck, as will be seen by reference to figure 3. It is decidedly oblong in shape and has the suckers grouped into a pair on each side.

As stated at the beginning of this paper, in spite of certain discrepancies in measurements between this worm and those described

by Guyer, the anatomical features which this worm has in common with *Taenia confusa* leave little room for doubt that it should be referred to that species.

If, when *Taenia bremneri* becomes better known, it shall prove to be identical with *Taenia confusa*, as there appears every reason to believe is the case, judging from our present meager knowledge of it, there will be little room for doubt but that *Taenia confusa*, like *Necator americanus*, and other noxious parasites, was brought to America from Africa with the slaves.

BIBLIOGRAPHY

- Guyer, M. F. 1898.—On the Structure of *Taenia confusa*. Zool. Jahrb., Syst., 11:469-492.
- Stephens, J. W. W. 1908.—Two New Human Cestodes and a New Linguatulid. Ann. Trop. Med. and Parasit., 1:549-556.
- Ward, H. B. 1896.—Report of the Zoologist, Ann. Report Nebraska State Bd. Agr. for 1895, 257-272.
- 1896.—A New Human Tapeworm. West. Med. Rev., 1:35-36. Copied in Amer. Monthly Micr. Jour., 18:305-307.
- for 1896, 173-189.
- 1897.—Note on *Taenia confusa*. Zool. Anz., 20:321-322.
- 1897.—Report of the Zoologist. Ann. Report Nebraska. State Bd. of Agr.

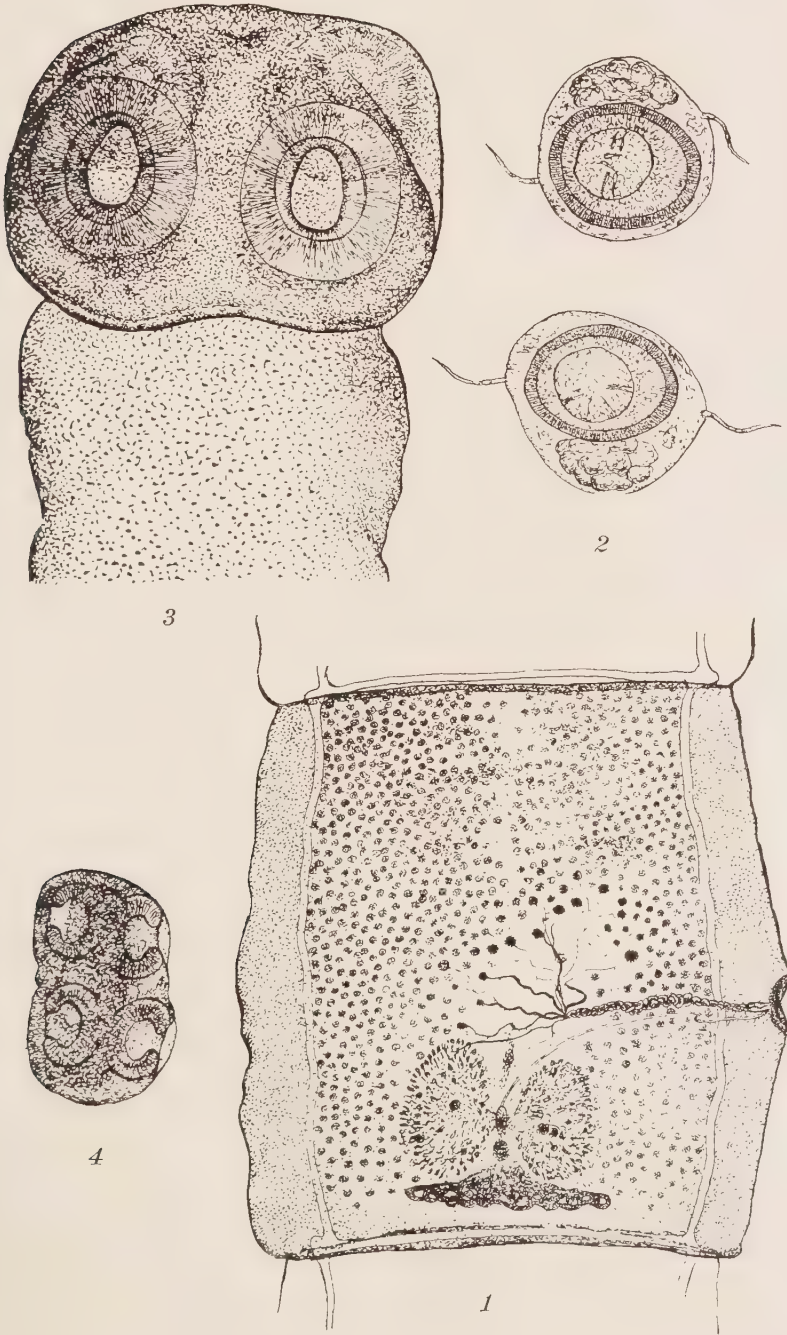
EXPLANATION OF PLATE VIII

Fig. 1.—Proglottid of *Taenia confusa*, a little past sexual maturity, showing general arrangement of organs, except uterus, which is very indistinct in this proglottid. Note very distinct vasa efferentia, and enlarged deep staining testes near the end of the vas deferens. $\times 7$.

Fig. 2.—Uterine eggs of *Taenia confusa*. $\times 500$.

Fig. 3.—Scolex of *Taenia confusa*, viewed on broad face. $\times 50$.

Fig. 4.—Scolex of *Taenia confusa*, as viewed from anterior end to show oblong shape, and bilateral arrangement of suckers. $\times 30$.



A POSSIBLE INTERMEDIATE HOST OF *FASCIOLA*
HEPATICA L. 1758 IN NORTH AMERICA *

MARK F. BOYD

Liver fluke disease of sheep and cattle caused by *Fasciola hepatica* L. 1758 is cosmopolitan in its distribution. According to Hall (1917), in the United States this disease is fairly well established along parts of the Pacific, the Gulf and eastern Atlantic coasts. Francis (1891) has definitely outlined its distribution in Texas. He states that: "This well known parasite occurs in the livers of cattle, sheep and goats of Texas in sufficient numbers to cause great damage. The portion of the state permanently infected consists of the coast counties and river bottoms."

The complicated life cycle of the fluke was first worked out in Europe by Creplin, Weinland, Leuckart and Thomas, and is too well known to require repetition. They found the larval stages to be passed in a small snail, *Limnaea truncatula*. According to Stiles, Leuckart also showed that the redia, but not the cercaria, would develop in *Limnaea peregra*. He also states that Lutz observed that in the Hawaiian islands both *L. oahuensis* and *L. rubella* serve as intermediate hosts. Stiles (1894-95) pointed out that none of these closely allied species of snails are found in America, while fluke disease is found in both North and South America, and concludes that there is either on this continent some other species of snail which may act as intermediate host, or some of the species described in America must be identical with some of the above named forms. He also advanced the view that in North America suspicion would especially fall upon *Limnaea humilis*, Say.

So far as we are aware, observations of the intermediate stages (sporocyst, redia, cercaria) of *F. hepatica* have never been recorded from any snail in North America. *L. humilis*, as suggested by Stiles, has been generally regarded as the intermediate host, but the fact has not been established. In view of the importance of this well known parasite, it seems surprising that this uncertainty regarding its North American intermediate host has not been cleared.

From time to time sheep have been grazed on Galveston island, and fluke disease has appeared among them. It therefore appeared probable that a suitable intermediate host must exist upon the island. Collections of fresh water snails from ephemeral pools on the island

* Contribution Number 4, from the Laboratory of Bacteriology and Preventive Medicine, Medical Department, University of Texas.

revealed the existence of three species, which were identified by Mr. H. B. Hannibal of San Francisco as: *Limnaea humilis*, Say, *Physa fontinalis acuta*, Drap., and *Succinea grosvenorii*, Lea.

Owing to the general view that *Limnaea humilis* is the intermediate host, it appeared that infection experiments designed to clear up this point would be of value. A limited supply of the living ova of *F. hepatica* were secured through the kindness of Prof. A. C. Chandler of Rice Institute, collected from the bile ducts of cattle and sheep at the Houston abattoir. A collection of adult fresh water snails was made on the island in the middle of December and kept alive in an aquarium, with the hope that they would spawn, and provide an adequate supply of young snails. Both *L. humilis* and *P. fontinalis* were represented in the collection. The *Limnaea* died in about two weeks without spawning. On the other hand, the *Physa* survived considerably longer and by Dec. 27 had deposited several masses of spawn.



Figure a.—Sketch of living redia of *Fasciola hepatica*. Length 560 micra.

Figure b.—Dorsal and lateral view of shell of *Physa fontinalis acuta*, Drap. Length of scale 10 mm.

The egg masses were transferred to a beaker containing tap water and algae, which was kept at room temperature, and were all hatched out by the middle of January. After all the young snails had emerged, a heavy suspension of *F. hepatica* ova was added to the beaker on January 16. At that time there were approximately 100 active young *Physa* in the beaker, about 1 mm. in length. On January 27 it was observed that the majority of the snails were dead and there were less than two dozen survivors. The remainder were examined

at varying intervals for larval stages of *F. hepatica*, by crushing them on a slide under a cover glass in a drop of the aquarium water. The records of these examinations follow:

Date	No. Snails Crushed	Results of Examination
1/27/20.....	2	Negative
2/ 2/20.....	2	Negative
2/12/20.....	3	Negative
2/20/20.....	1	Found two rediae
2/27/20.....	3	Found three rediae in one snail
3/10/20.....	5	Found eleven rediae in one snail
3/12/20.....	4	Negative

All of the fluke ova employed were apparently fertile, as none were observed in the aquarium by February 12 with the operculum in place, showing that a miracidium probably emerged from each ovum.

The rediae were apparently in the digestive gland of the snail. The appearance of a single redia is shown in figure *a*. They were as actively motile as could be expected, since they were under compression. Contraction and expansion of the body was marked in all, together with contraction and expansion of the muscular pharynx. The blind gut was filled with cellular debris evidently derived from the digestive gland of the snail. When contracted they measured about 450 micra in length, and when expanded, about 560 micra. The space between the cuticle and gut contained several masses of germinal cells. In none were cercariae found.

It is to be regretted that we failed to secure a sufficient number of infected *Physa* so as to prolong the observations to determine whether cercariae would develop and emerge. As it is, the data presented do not enable us to conclude that the larval cycle can be completed in *Physa*, nor settle the question regarding *L. humilis*. Later in the season when we did secure a small brood of *L. humilis* from spawn, we were unable to secure any fluke eggs.

In this connection, some statements by Gilchrist (1918) are of interest. He states that in South Africa the commonest fresh water snail is *Physa (Isidora) tropica* and that in them are found abundant stages of a fluke very closely resembling those of the liver fluke. He also states that in Australia the intermediate host of the liver fluke is believed to be a species of *Physa*.

REFERENCES CITED

- Francis, M. 1891.—Liver flukes: The common fluke (*Distomum hepaticum*), a new species (*Distomum texanicum*). Bull. Texas Agr. Exp. Sta. 18:127-136, 8 pls.
- Gilchrist, J. D. F. 1918.—The life history of *Distoma luteum*, n. sp., with notes on some cercariae and rediae found in S. Africa. Parasitology, 10:311-319.
- Hall, M. C. 1912.—Our present knowledge of the distribution and importance of some parasitic diseases of sheep and cattle in the United States. Circ. Bur. An. Ind., 193:419-463, 2 pls.
- Stiles, C. W. 1894-95.—The anatomy of the large American fluke (*Fasciola magna*), and a comparison with other species of the genus *Fasciola*, s. st. Jour. Comp. Med. and Vet. Arch. 15:161-178, 225-243, 299-313, 407-417, 457-462.
- Stiles, C. W. 1898.—The flukes and tapeworms of cattle, sheep and swine, with special reference to the inspection of meats. Bull. Bur. An. Ind., 19:11-136, 124 figs.

DIBOTHRIOCEPHALUS TÆNIOIDES LEON, A NEW
CASE IN ROUMANIA *

N. LEON

The helminth which forms the object of this note was sent by Doctor Bacaloglou, Professor of Clinical Medicine in our faculty, and accompanied by the following record: "Mr. P., 60 years old, director of a bank, had suffered for two months from gastro-intestinal troubles. He is habitually of good health, not syphilitic, alcoholic, nor given to smoking. The urine at present contains neither sugar nor albumen.

"In spite of the efforts of several physicians, he continued to suffer with vague intestinal pains, and especially a painful morning diarrhea. On some days he had five or six evacuations, accompanied by particles of solid mucus and even of whitish masses like coagulated white of egg. Milk diet to which he was submitted originally, aggravated all these symptoms. I discontinued the milk and put the patient on a diet composed of gruels, soups, and marmalades. I prescribed for him 0.50 cg. of calomel, and following this capsules with betol (1.50 gm. daily with salicylated bismuth). Thereupon he expelled a long fragment of a broad tapeworm. As it did not possess the head, I had him take two days later 6 grams of extracts of male fern. In place of obtaining the remainder of the worm, which perhaps was lost with the fecal matter, the patient expelled a large specimen of *Ascaris lumbricoides*.

"I add that the general condition of the patient, in spite of the gastro-intestinal troubles which had persisted for two months, was good. He had none of the anemia described by authors for carriers of the broad tapeworm—anemia which furthermore did not exist among other patients that I have cared for in that disease. A fortnight after the expulsion of the two parasites, I revisited the patient. He was happy at the disappearance of the diarrhea. The phenomena of mucomembranous entero-colitis are certainly related to the parasites that he harbored."

The worm lacks the head, neck and a good portion of the chain with young rings. The length of the part evacuated is 82 cm., but the widest segments measure hardly 6 mm. The color is ashy yellow, and the segments are very delicate. The form of the segments varies according to age. The youngest, that is to say those closest to the head, are a little broader than long, at most one and one-quarter, but

* Laboratoire d'Histoire naturelle médicale et Parasitologie de l'Université de Jassy, Roumanie.

never several times broader, as in the case of *Dibothriocephalus latus*. The joints change in a gradual manner into a quadrate type equal in both dimensions. At the end the oldest joints are longer than broad, and all the longer because they are more advanced in age. In comparing this specimen of Professor Bacaloglou's with the specimen that I described (No. 2 in the *Centralblatt f. Bakt., I Abt. Originale*, 1916), I determine that the two individuals are the same species. The

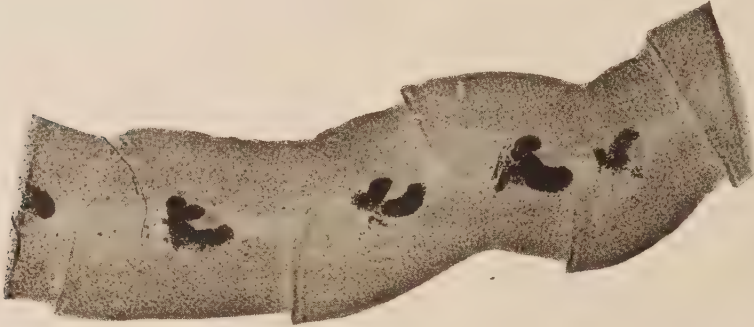


Fig. 1.—*Dibothriocephalus taenioides*. Fragments of chain.

common characteristics which distinguish them from *Dibothriocephalus latus* are not anomalies, as I thought at the beginning, since the anomalies which have been observed among the Bothriocephalids, such as intercalated segments, fenestrated rings, scaly segments, etc., are isolated, or even if they form a portion of the chain, it is relatively

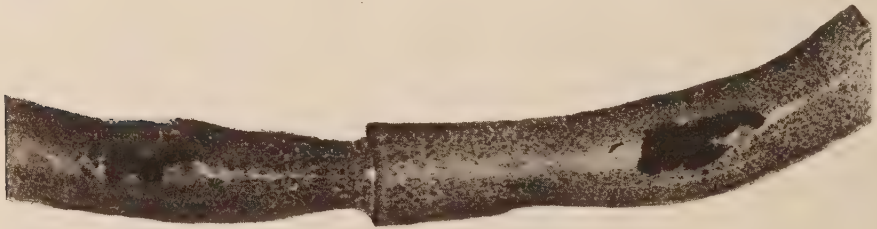


Fig. 2.—End portion of the chain.

very short in relation to the length of the normal worm. The abnormal rings are always situated between other rings of the normal series. The coils of the uterus, which show themselves easily on account of the transparency when they are full of eggs, are two or three in number, and their arrangement takes a characteristic form, bi- or tricornuate (Fig. 3). Even under the naked eye and at a distance one recognizes that the worm is not *Dibothriocephalus latus*. In that species the coils in general number five on each side. They show

themselves under the classic appearance of a rosette, while in *Dibothriocephalus taenioides* they are, as I have said above, bi- or tricornuate.

The characteristics which necessitate the creation of this species under the name of *Dibothriocephalus taenioides* are the following:

(a) *Form of Proglottids*.—The ripe joints are always longer than broad, and the others follow on with the most striking regularity as in *T. solium* or *T. saginata*, first those a little broader than long; then those which are quadrate and following them such as are longer than broad.

(b) *Size of Segments*.—The largest segments of *Dibothriocephalus taenioides* hardly reach 6 mm., but the major part are narrow as in *Dibothriocephalus parvus* Stephens; yet they differ from segments of the latter in that these, although very narrow, are very short, while the segments of *Dibothriocephalus taenioides*, while narrow, are long.

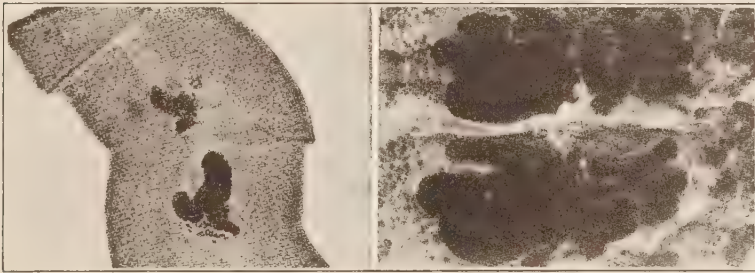


Fig. 3.—At left, *Dibothriocephalus taenioides*, and at right, *Dibothriocephalus latus*, photographed to compare uterine rosettes.

(c) *Form of the Uterine Rosette*.—In *Dibothriocephalus latus* the uterine coils are five on each side, forming the characteristic uterine rosette, while in *Dibothriocephalus taenioides* uterine coils filled with eggs number only two or three.

(d) *Musculature*.—The musculature, both longitudinal and circular, is markedly reduced, so that as a result *Dibothriocephalus taenioides* is very slender.

(e) *The color* is a characteristic ashy yellow.

The preparations are preserved in the collection of the Laboratory of Parasitology of the Faculty of Medicine at Jassy.

A NEW COURSE FOR MIGRATING ANCYLOSTOMA AND STRONGYLOIDES LARVAE AFTER ORAL INFECTION

SADAO YOSHIDA

Pathological Department, Osaka Medical College

OSAKA, JAPAN

The modes of infection and course of migration for *Ancylostoma* and *Strongyloides* have been accurately and decisively investigated by various helminthologists old and new; especially recent works by Looss and Fülleborn have decided almost all problems in the subject so fully that no further investigations are needed.

Ancylostoma and *Strongyloides* larvae are believed to infect in two ways, through the skin and the mouth, the former being the more prevalent manner of infection. The course of the migrating larvae in the host is essentially the same in both cases of skin and of oral infection. Sooner or later they migrate into the lungs by means of blood vessels or sometimes by the lymph system; then the majority of the larvae in the lungs pass through the trachea, esophagus and stomach to the small intestine where they grow into the adult form; but a few of them migrate from the lungs to the intestinal wall by way of the blood vessels, passing through the pulmonary vein, heart and mesenteric arteries, whence they penetrate into the canal of the intestine where they become mature.

This is the universally recognized course of the larvae in the body of the host. In connection with my study on the migration of ascarid larvae in the body of their host, it occurred to me that the well defined course of *Ancylostoma* and *Strongyloides* larvae in the host although the principal one, may not be the only one. The following experiments will serve to test not only the new course of migrating *Ancylostoma* and *Strongyloides* larvae, but prove definitely my proposal regarding the migration of ascarid larvae.

For the experiments *Ancylostoma* larvae were cultured from eggs from human feces and filariform *Strongyloides* larvae were obtained from a culture of eggs from the feces of some monkeys.

Exp. 1. At 5 p. m. on July 17, 1918, two guinea-pigs, A and B, were fed with the *Strongyloides* larvae cultured for nine days. Animal A was killed at 1 p. m. on the next day, twenty hours later. Two specimens of active larvae were found in the pleural cavity and three in the abdominal, but none in the lungs.

Exp. 2. At 9 a. m. on July 24, a guinea-pig was fed with the *Strongyloides* larvae cultured seven days and it was killed at noon on the next day, twenty-

seven hours later. Six specimens were found in the abdominal cavity, two in the pleural and two in the left lung but none in a piece of the liver. Pancreas unexamined.

Exp. 3. Animal B of Exp. 1 was killed at 9 a. m. on July 19, forty hours after feeding. Two living larvae were found in the abdominal cavity and three in the lung which was slightly blooded, also three in a piece of the liver and one in the pancreas.

Exp. 4. At 11 a. m. on July 19, a guinea-pig was fed with the *Strongyloides* larvae cultured eight days and at 11 a. m. on the twenty-third, ninety-six hours later, it was killed. Abdominal examination was interfered with on account of bleeding by cutting the liver carelessly. Only one specimen in the pleural cavity, three in the right lung and two in a piece of the liver. Pancreas unexamined.

Exp. 5. At 11 a. m. on July 19, a guinea-pig was fed with the *Ancylostoma* larvae cultured eight days and at 1 p. m. on the next day it was killed, twenty-six hours after feeding. Two worms were found in the pleural cavity, five in the abdominal cavity, many in the lungs, a few in the liver and three in the pancreas.

Exp. 6. At 9 a. m. on July 24, a guinea-pig was fed with the *Ancylostoma* larvae and at 3 p. m. on the next day it was killed, thirty hours later. Three were in the pleural cavity, four in the abdominal, many in the lungs, two in a piece of the liver and one in the pancreas.

Exp. 7. During three hours from 9 to 12 a. m. on July 22, *Strongyloides* larvae six days old were smeared from time to time on the abdominal skin, where the hair was cut off closely and shaved, and the animal was fixed on the holder until 5 p. m. Then it was put in the cage after the smeared part had been cleaned. At 9 a. m. on the next day, about twenty-four hours later, it was killed. Four were found in the abdominal cavity and two in the pancreas, but none in the lungs, pleural cavity or in one-half of the liver.

From the above experiments one may easily assume the two facts that: 1, *Ancylostoma* and *Strongyloides* larvae introduced into the alimentary canal of the feeding animal may appear in the abdominal and pleural cavities at least twenty-four hours later, and may penetrate into the liver, pancreas and lungs; 2, *Strongyloides* larvae smeared on the shaved skin of the abdomen may pierce through the abdominal wall and appear in the abdominal cavity or penetrate into the organs of the cavity in about twenty-four hours.

The piercing power of *Ancylostoma* and *Strongyloides* larvae is commonly recognized without which skin infection or further migration into the intestinal wall by the larvae infected, is not easily explained. The above experiments also show the fact clearly that the larvae may pierce through the skin or the wall of the alimentary tract.

Appearance in the pleural cavity of larvae introduced into the alimentary canal is explained in two possible ways: 1, the larvae may pierce through the esophageal wall and reach the pleural cavity and 2, the larvae may pierce through the gastral or intestinal wall to enter first the abdominal cavity, and thence proceed to the pleural cavity by passing through the diaphragm. Previous authors fre-

quently reported finding larvae in the esophageal wall. So the first way may be supposed to exist. From the result of the above experiments as well as from my conclusion obtained by experiments on the migration of ascarid larvae, it seems to me, however, that the second way is the more common and usual course of the larvae reaching the pleural cavity.

Thus I am strongly inclined to believe in the piercing power of larvae in their migration, during which they enter the abdominal cavity by boring the alimentary wall and thence proceed to the pleural cavity by passing through the diaphragm and lastly penetrate the lungs from the surface, as in the case of ascarid larvae. This will be probably a new course for the migration of *Ancylostoma* and *Strongyloides* larvae in the body of the host.

Fülleborn described finding *Strongyloides* larvae not only in the lungs but in the liver and kidneys of a dog fed with the larvae and explained the appearance of the larvae by their migration by way of blood vessels. His theory may be true. However, I believe it is also reasonable to explain the presence of larvae in these organs by direct penetration from the surface by means of their own piercing power. Some larvae in these organs may certainly be considered to have penetrated from the abdominal cavity which they reached from the intestine by passing through its wall. The larvae in the pancreas of my case may also be understood to have entered the organ from its surface, not by way of blood vessels.

There may be hard places for the larvae to pass from the intestine to the kidney, if they go by means of blood circulation. Thus it will be harder for the larvae to reach the kidneys and pancreas from the intestine by way of blood vessels than by the direct penetration into the organs from their surface by their own piercing power.

Several authors report cases in which they found many larvae in the tissues of the intestinal wall instead of in the blood vessels. Might this not be a case showing their penetration through the tissues? And some investigators described relatively scant occurrence or even absence of larvae in the liver of the infected animal whereas the lungs were heavily invaded. This may, of course, be partly attributed to an incomplete examination, but is also easily explained by assuming the direct migration of the larvae.

I am inclined to believe that the direct penetrating migration of *Ancylostoma* and *Strongyloides* larvae in passing through the tissues of the host, is a new course, additional to the old well known route by the blood vessels.

A METHOD OF CONCENTRATION OF PARASITIC EGGS IN FECES

WILLIAM H. GATES

The microscopic examination of feces for eggs of intestinal, hepatic and some other parasites depends largely for success upon discarding unnecessary fecal matter and concentrating eggs into as small a space as possible. Many methods have been devised (see Hall, Bull. 135, U. S. Dept. Agric.). A modification used by the author is as follows:

After straining through a seive a large quantity of material, or using a smaller quantity without this, feces are centrifuged first with water to wash off surplus lighter material, and later with sodium chlorid, or better, calcium chlorid solution, sp. gr. 1.250, to remove the bulk of the material and float the eggs practically free from sediment. The top one or two cubic centimeters are then removed with a pipette, drawing chiefly from the rim of the meniscus, and centrifuged again with water, which throws the eggs to the bottom. The water is then poured off, leaving *all* of the sediment in the bottom. This sediment is agitated vigorously by holding the tube in the closed hand and pounding on the table. This stirs up all or nearly all of the eggs which may have stuck to the bottom, though a few eggs cannot be removed except with a brush. The sediment is quickly poured into a small dish. The centrifuge tube is rinsed out by squirting water forcibly into it and this also is poured immediately into the dish. The eggs settle rather rapidly and are loosened from the bottom by forcing a little water around the edge to produce a slight whorl. Then before the eggs have a chance to settle, agitate the dish in the same circular direction so that the water tends to form a vortex, gradually diminishing the motion until it is hardly more than a jar. Practically all of the eggs will be found to have settled within a very small field.

For gross examination with the low powers, the eggs may be left in the dish and examined directly. To examine more carefully, under a high degree of concentration, draw up with a pipette a small quantity of water from the center of the mass of eggs; hold this vertical and steady for a half minute or so. Most of the eggs will settle, so that a single drop forced out on the slide will contain nearly all, if not all, of the eggs drawn up into the pipette. For still further concentration, allow the eggs on the slide to settle, and then with a blotter or lens paper very carefully remove a portion of the water from the top of the drop and add another drop. If repeated with care, a large mass of eggs may be collected in the space of a cover slip. This is especially satisfactory if the eggs have been in alcohol for the alcohol will evaporate, leaving the eggs in the center.

BOOK REVIEW

MANUAL OF TROPICAL MEDICINE. By Aldo Castellani, C.M.G., M.D., M.R.C.P. and Albert J. Chalmers, M.D., F.R.C.S., D.P.H. Third Edition, New York, 1920, William Wood and Company. 2436 pages, 909 text figures and 16 colored plates.

The appearance of this magnificent volume was so closely coincident with the news of the sudden and unfortunate death of the junior author that the work stands in a very real sense as a monument to his ability and industry, in every way worthy of a career which, though brief, was one of marked achievement.

While the second edition of this manual has been out of print for some years, there has been a natural delay in the preparation of the new edition due to the war and its consequent difficulties in many directions. Despite these, the authors have succeeded in producing a work that is in every way worthy of high praise. So far as mere size goes, the new material introduced has expanded the volume fully one half and the illustrations by an equal amount. Nor has this been all, since the use of smaller type for historical and subsidiary items has allowed the introduction of still more new matter. The work is not only admirably comprehensive without being diffuse but the index, which covers 152 pages, and is in every way well constructed, makes the book useful for rapid reference as few of its size really are.

Much new matter has been added to chapters on plague, fevers, influenza, cat bite fevers, typhus, etc., and the additions incorporate in large part the most recent studies on the relations of animals to these diseases. Entirely new chapters on war zone fevers, diagnosis of tropical fevers, tropical poisonings, myiasis and allied conditions, among others, give evidence of the careful efforts exerted to bring the work fully up to date and to enhance its practical value for the worker in the tropics. The rather scanty treatment of tsutsugamushi disease, so important in Japan, is no doubt due to the inaccessibility of the literature, some of the most significant of which is also of very recent date.

Part I of this work is introductory and contains chapters on the history of tropical medicine, tropical races, climatology, foods, diseases, fitness for tropical life. Part II, the classification of diseases in the tropics, has separate sections covering physical causes, chemical causes and parasites, both animal and vegetable. Part III, the diseases of the tropics, has sections on fevers, general diseases, and systemic diseases. Each of these sections is sub-divided on the basis of causation. It is interesting to note that under the fevers thirteen chapters are devoted to those probably of protozoal origin and carried by animals, three chapters to those of bacterial origin, of which two are related to animal carriers, and only four chapters to other types of fevers. In the section on general diseases, animal causation is held responsible for two-thirds of the diseases listed. No better evidence could be given of the tremendous rôle played by animal parasites in tropical diseases. And no one can examine a work like this without being profoundly convinced of the importance of thorough study of animal life for the worker in tropical medicine.

Attention may properly be confined here to those details that fall within the scope of parasitology, although other parts of the book are not only of great interest to workers in medical zoology but are full of valuable material bearing directly upon their subject.

The changes in section C, Parasites, which embraces 740 pages, are so great that one may properly regard this part as a new monograph on the topic. Especially worthy of note is the introduction of a new chapter entitled

The Animal Carriers of Disease, which discusses in thoro fashion the problems connected with animal carriers, relation of hosts, the question of disease reservoirs, contrasts between different types of insect borne diseases, complex life histories of worm parasites and the contrasting relations of bacterial diseases to animal carriers.

Under Animal Parasites the forms are classified in accordance with the zoological system. This has been carefully applied by an author who is not only well acquainted with the animals, but is familiar with the necessary restrictions that have grown up in the subject and that must apply to those who, tho not zoologists, find it necessary to utilize technical material in the field. It would be a piece of good fortune for zoological research and for the proper treatment of parasitic diseases if every worker in this field were compelled to study carefully the material presented in this section of the volume. The authors have made a good move towards the substitution of uniformity for the chaos that exists in medical literature by following, for the names of parasites, as in previous editions, the rules of the International Zoological Congress. It would be hard to find a clearer and more convincing statement of the case concerning nomenclature of parasites than that given in a half page in the preliminary paragraphs under the division on animal parasites.

Undoubtedly the most difficult phase of this topic is that which treats of protozoal parasites. The organisms are so minute, the differences so difficult to determine, and many types are to the untrained observer so thoroly identical, that even in more technical publications there is great confusion with reference to the number of forms that may rightly be distinguished as independent types of organisms. It is a little unfortunate that the author could not have had for consideration the recent comprehensive study of the *Amoeba Living in Man* by Dobell, reviewed in a recent number of this JOURNAL. The differences between the two accounts are sufficient to be the source of serious confusion to those who have not made an intensive study of the field. Specialists recognize, however, that such differences are inevitable in early work on any topic, and it is not too much to say that we have only just begun to get a grasp on these forms.

There is no synopsis of the protozoa parasite in man in which the topic is presented so completely and at the same time so concisely as in the volume under revision. Its fairness in presenting various aspects of disputed questions and its completeness make the record invaluable for those who are trying to work on these little-known organisms. Chalmers includes the *Spirochaetes* along side of the *Trypanosomes* under the *Binucleata* and thus conforms to the opinion of most zoologists, altho he departs from the views of many pathologists and especially of the bacteriologists who would include them with the bacteria. While it is undoubtedly too early to reach a final conclusion on so complex and obscure a question, yet for the purposes of this volume and of work in tropical medicine the setting given these forms is abundantly justified.

It is indeed in the group of flagellates that the greatest confusion obtains at the present moment. Not only do these forms lack in recognizable morphological characteristics but they are so minute and include so many types that are merely developmental stages that even the trained observer is inclined at times to abandon the attempt to interpret forms he finds. In this field Chalmers has himself worked so long and successfully that the scientific world is fortunate in having him to guide its progress thru the intricate mazes of the problem. And many new genera make their appearance for the first time in these pages in proper systematic relations.

Much new material has been included in the section on the *Sporozoa* and the work gives the best available survey of this which is another little known field. The treatment of parasitic worms may also be commended for its com-

pleteness and for the judgment exercised in introducing new material. One finds little to criticize; perhaps the accounts of the life history of *Ascaris* and of *Schistosomes* are the most imperfect.

The short chapter on the leeches is rather unsatisfactory and the single figure given is really little more than a joke, but this group is no doubt of all the least significant for the worker on tropical diseases. The Arthropod chapter is well handled, if one excuse the omission on grounds difficult to suggest in view of the completeness of the work otherwise, of the splendid work done by Howard and his colleagues on mosquitoes.

Some minor points deserve passing criticism; the authors use, for instance, in a synopsis the names *Platyhelminthia* and *Nemathelminthia* which appear in other places as *Platyhelminthes* and *Nemathelminthes*. Such variant forms, easily recognized by the specialist, are apt to be serious stumbling blocks for those not trained in zoological lines. Tho some of the misprints of the earlier edition have been corrected, others still remain not merely in the names of well-known scientific workers and journals, but in a few scientific names where it might be difficult to recognize in *Collyrichum* and *Hocyalomma* the correct forms *Collyriclum* and *Hyalomma*. It is unfortunate to find such terms as *Rattenkönig cercariae*, which is not even a correct citation of the German term and is readily translatable by the equivalent *Gorgonocephaloid*.

On the whole the figures are admirable and abundant. The colored plates are very well done and the photographs of eggs by Bell are both new and thoroly desirable, altho some of them have come out in the printing rather indistinctly and the value of the representations (p. 626) of such as are of doubtful identification is seriously reduced in that no indication is given of the magnification or actual size. The literature lists have unfortunately not been revised and stand almost everywhere exactly as in the earlier edition, altho much new and very important material has been added to the text. This throws a heavy burden upon the student who wishes to refer to the recent contributions in order to follow up more fully an individual problem. Typographical errors seem to be more frequent in authors' names and in the designation of periodicals than elsewhere, altho it must be confessed that the percentage of such errors is fortunately small.

It would be hard to record the excellencies of the work with the same fullness with which the minor errors have just been reviewed. From cover to cover the volume shows adequate knowledge and control of the field. Its conciseness and clearness of presentation cannot be too highly commended. The fulness with which it includes most recent work, and the freedom from bias in dealing with moot questions and unsolved problems are admirable features that must be emphasized in closing this review. The work is a storehouse of splendidly arranged material, and as such is indispensable to every worker interested in any phase of medical zoology.